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Residual Effectiveness of Chlorinated Hydrocarbons for Control of the Imported Fire Ant[1]

G. H. Blake, Jr., W. G. Eden, and K. L. Hays, *Alabama Polytechnic Institute, Auburn*

Abstract

Experiments were conducted in Alabama from 1953 to 1958 on control of the imported fire ant, *Solenopsis saevissima* v. *richteri* Forel. Chlordane as a dust and in granular formulation, aldrin in emulsion spray and granular formulation, dieldrin in emulsion spray and granular formulation and in fertilizer mixture, and heptachlor in granular formulation and fertilizer mixture were applied in late winter or early spring in a series of experiments to permanent sod lands. Two pounds of heptachlor or dieldrin, or 4 pounds of chlordane per acre, when broadcast as granules, were highly effective for control of the ant and afforded good control for a period of 3 to 5 years. In one experiment, granulated dieldrin was effective for a longer period than a dieldrin emulsion spray. Lower dosages of the insecticides gave good control for 1 year after treatment, but they were ineffective thereafter. When the insecticides were mixed with fertilizer, approximately the same degree of fire ant control was obtained as when the insecticides were used alone. Chlordane mound treatment killed the ants in the treated mounds but did not prevent reinfestation. When broadcast treatments lost their effectiveness, the number of mounds per acre was greater than on the non-treated areas, but the new mounds were smaller in size. Based on 1-year results, airplane application of 5% or 10% heptachlor at a rate of 2 pounds of technical material per acre were equally effective for control of the imported fire ant. Soil type appeared to have no effect on fire ant control.

The rapid spread of the imported fire ant, *Solenopsis saevissima* v. *richteri* Forel, over the southeastern United States in the past few years has created much interest.

Results of research begun in 1949 on control of the insect in Alabama and information on its introduction into the United States were given in previous papers by Arant & Eden (1949) and Eden & Arant (1949). Good control of the ant was obtained with broadcast treatments of 4 pounds of chlordane per acre. Several insecticides were effective against the ant when used as mound treatments. Research in Mississippi by Green (1952) also showed that chlordane was effective as a mound treatment, but such treatment did not usually eradicate the ant from an area. Fumigation of mounds gave poor control, and the use of baits was not effective. Other information on control of the imported fire ant has been published by Eden & Garrett (1955) and Blake (1956).

The status of the imported fire ant in Argentina was reported by Hays (1958); the insect was not considered an economic pest in its native environment. A description of the effects of the sting of the ant on man, as well as information on the biology and control of the insect in Georgia, was published by Favorite (1958).

Additional experiments on control of the imported fire ant in Alabama were begun in 1953. Results of this research through February, 1958, are presented in this paper.

Methods and Procedure.—A series of experiments on control of the imported fire ant was begun in March, 1953, on heavy clay soils of the Black Belt. In all of the experiments the treatments were applied in late winter or early spring, when there was a minimum of foliage on the ground. The first experiment included an untreated check, chlordane as a mound treatment, aldrin as an emulsion spray, and dieldrin as an emulsion spray and as granules. The treatments were unreplicated in plots 2 to 5 acres in size. A 10% chlordane dust was applied to individual mounds with a rotary-type hand duster at 2 to 3 ounces of dust per mound. The mounds were raked down prior to the application, and the dust was applied to the mound and to the ground surface approximately 3 feet around the mounds. Aldrin and dieldrin were applied as broadcast treatments at 2 pounds of technical material per acre. Sprays were applied with a power sprayer equipped with a 16-foot boom (10 Spray-jet 80.2 nozzles placed 19 inches apart). The sprayer was calibrated to deliver 16 gallons per acre at 40 to 45 pounds pressure. Four per cent granulated dieldrin was applied at 50 pounds per acre with an Ezee-Flow fertilizer distributor. To deliver 50 pounds of granules, a setting for 25 pounds of fertilizer per acre was used. The mounds were not mechanically disturbed prior or subsequent to the insecticidal application.

The second experiment was begun on March 9, 1954. Treatments in the experiment included an untreated check, dieldrin and heptachlor at 0.5, 1.0, and 2.0 pounds per acre, and chlordane at 4 pounds per acre. Treatments were duplicated in long, narrow plots with plot areas being 0.75 and 1.0 acres, respectively, for the replicates. The insecticides were broadcast with a fertilizer drill, except one replication (the 0.5 pound rate of dieldrin) was applied with a Cyclone seeder. Dilute granulated formula-

[1] Partial cost of publication of this paper was met by the Department of Zoology-Entomology, Alabama Polytechnic Institute School of Agriculture and Experiment Station System. Accepted for publication October 13, 1958.

tions were used to ensure good coverage. A 2% formulation of dieldrin was used for the 0.5- and 1.0-pound dosages, and a 5% formulation for the 2-pound rate. For the respective dosages of heptachlor, 1, 2, and 4% granulated formulations were used. Chlordane was applied in a 10% granulated formulation.

In 1955 heptachlor and dieldrin were applied on February 4 and 5 as broadcast treatments in mixtures with fertilizer. Dilute granulated formulations of the insecticides were mixed with the fertilizer at the fertilizer plant in ratios to give 0.67 and 2.0 pounds of heptachlor, and 1.1 pounds of dieldrin in the quantity of fertilizer (300 pounds) to be applied per acre. The materials were applied with a fertilizer drill. Treatments were unreplicated in large plots varying in size from 14.5 to 29.5 acres.

Three experiments were begun in March, 1957. In two of them, the treatments were applied with a fertilizer drill, whereas in the third experiment the insecticides were applied by airplane. In the two experiments in which the insecticides were applied with ground equipment, the treatments were heptachlor, dieldrin, and aldrin at 2 pounds, and chlordane at 4 pounds per acre. One experiment was conducted on heavy clay soil and the other on sandy loam. Treatments in these experiments were replicated four times in randomized blocks of plots.

In the experiment in which the insecticides were applied by airplane, heptachlor was used at 2 pounds per acre, in 5% and 10% granulated formulations. Treatments were unreplicated in 30-acre plots.

In all of the experiments, the treatments were evaluated by determining the number of active imported fire ant mounds on the entire plot areas at intervals following treatment. Mound counts were made yearly in late winter or early spring when a minimum of vegetation was on the ground and the mounds could be seen easily. In all experiments the numbers of mounds on the areas were counted prior to treatment to determine the relative population of ants in the various areas.

RESULTS.—The numbers of imported fire ant mounds per acre for each of the 5 years following treatment in 1953 are given in table 1. The number of mounds per acre in the nontreated area was fairly constant for the period of the experiment, averaging approximately 20 per acre. The granulated dieldrin afforded complete control for 3 years and was still effective after the fourth and fifth years. However, there were indications that the ants were becoming re-established in the area. The dieldrin spray was highly effective for 2 years. Although the treatment gave good protection during the third year after treatment, the mounds by the fourth year had increased to essentially the same number as in the untreated area. The aldrin spray afforded fair control for 1 year, but after the second year the number of mounds per acre was greater than on the untreated area. Chlordane mound treatment killed the ants in the treated mounds but did not prevent reinfestation. The number of mounds per acre remained at about the same level as in the untreated area.

Table 1.—Active mounds of the imported fire ant on large plots at intervals after treatment in 1953.

TREATMENT (2 LB./A.)	NUMBER OF MOUNDS PER ACRE AT INDICATED YEARS AFTER TREATMENT				
	1	2	3	4	5
Untreated	25	15	20	21	18
Dieldrin, granules	0	0	0	2	4
Dieldrin, spray	0	1	6	16	20
Aldrin, spray	8	18	50	34	—
Chlordane,[a] dust	16	9	17	29	—

[a] Two to three ounces per mound.

Table 2.—Average number of active mounds of the imported fire ant on plots at intervals after treatment with granulated insecticides in 1954.[a]

TREATMENT AND POUNDS PER ACRE	NUMBER OF MOUNDS PER ACRE AT INDICATED YEARS AFTER TREATMENT			
	1	2	3	4
Untreated	26.0	29.5	30.5	33.0
Dieldrin, 0.5	12.5	41.5	46.0	44.0
Dieldrin, 1.0	3.0	35.0	52.5	39.0
Dieldrin, 2.0	2.5	11.5	15.0	17.0
Chlordane, 4	0	6.0	17.5	13.5
Heptachlor, 0.5	0	20.0	38.5	30.0
Heptachlor, 1.0	0.5	30.0	22.5	22.0
Heptachlor, 2.0	0.5	1.0	4.0	2.5

[a] Average of two replications.

Results of the experiment begun in 1954 are presented in table 2. Again, the number of mounds on the untreated areas remained at about the same level from year to year. Two pounds of heptachlor was highly effective for the 4-year period. The lower rates of heptachlor were highly effective for 1 year, but, by the end of the second year, the number of active mounds had increased to approximately the same as that on the untreated area. Chlordane was completely effective for the first year, but had begun to lose its effectiveness by the second year after treatment. Dieldrin at rates of 1.0 and 2.0 pounds per acre in this experiment was highly effective for 1 year. The 2-pound dieldrin treatment was less effective after the 1st year; the 1-pound rate was ineffective after 1 year. It is difficult to understand the performance of the 2-pound rate of dieldrin in this experiment, since it was effective for 5 years in the previous experiment. The ½-pound dosage of dieldrin gave some control of the ants for the first year but was ineffective thereafter.

Results of the experiments with insecticide-fertilizer mixtures for imported fire ant control are given in table 3. Where the insecticides were mixed with the fertilizer, approximately the same degree of fire ant control was obtained as when the insecticides were used alone. The

Table 3.—Number of active mounds of the imported fire ant on large plots at intervals after treatment in 1955 with insecticides in fertilizer mixtures.

TREATMENT AND POUNDS PER ACRE	NUMBER OF MOUNDS PER ACRE AT INDICATED YEARS AFTER TREATMENT		
	1	2	3
Untreated	21.0	33.0	31.4
Heptachlor, 0.68	6.1	42.2	—
Heptachlor, 2.0	0	1.2	5.7
Dieldrin, 1.1	5.0	46.8	—

lower dosage of heptachlor (0.68 pound per acre) and the dieldrin treatment (1.1 pounds per acre) gave good fire ant control for 1 year but were ineffective thereafter. The 2-pound rate of heptachlor was effective for the 3-year period.

Results of the two experiments begun in 1957 on heavy clay and sandy loam soils revealed that plots treated with 2 pounds of aldrin, dieldrin, or heptachlor, or 4 pounds of chlordane per acre contained 1 year after treatment an average of one mound or less per acre (table 4). The untreated areas averaged 23.3 and 25.5 mounds per acre on the heavy clay and sandy loam soils, respectively. Thus, 1 year after treatment soil type had no effect on the performance of any of the insecticides.

In the experiment on control of ants with 2 pounds of heptachlor applied as granules by airplane, 20 pounds of 10% granules and 40 pounds of 5% granules were equally effective. Both treatments resulted in highly effective fire ant control 1 year after treatment, resulting in one mound or less per acre.

Mound counts made on the experimental areas prior to treatment revealed that the average number of mounds per acre from year to year varied from 13 to 31. The mound counts from practically all of the older experiments revealed that when broadcast treatments lost their effectiveness, the number of mounds per acre on treated areas was greater than on the untreated plots. The new mounds though greater in number were smaller in size. Over a period of years the mounds became larger and the number of mounds per acre was again approximately equal to the number on the untreated areas.

Table 4.—Active mounds of imported fire ant on two soil types after treatment with low concentrate granules in 1957.[a]

Treatment and Pounds per Acre	No. of Mounds per Acre 1 Year After Treatment	
	Clay Soil	Sandy Loam Soil
Untreated	23.3	24.5
Heptachlor, 2	0.1	0.3
Dieldrin, 2	0.7	0.2
Aldrin, 2	1.3	1.1
Chlordane, 4	0.7	0.0

[a] Average of four replicates.

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Soil Fumigation to Control Weevil Grubs in Strawberries[1]

Paul M. Eide[2]

Abstract

Ethylene dibromide was a practical fumigant against *Nemocestes* (*Geoderces*) *incomptus* (Horn), *Sciopithes obscurus* (Horn), *Brachyrhinus sulcatus* (F.) and *B. ovatus* (L.) in strawberries at dosages as low as 1.6 gallons per acre applied to only the rows. A dosage of 8.6 gallons did not injure the plants. A mechanical applicator which applied any desired amount of insecticide uniformly without appreciable injury to the plants was devised. Nemagon, applied with a hand injector, was not successful in these tests, nor was Dipterex when used as a drench. Strawberry plants were killed by Vapam when applied as a drench.

The incorporation of insecticides into the surface layer of soil in fields to be planted to strawberries was first recommended for control of *Brachyrhinus* sp. in 1952 (Eide 1952). This has become a general practice. Most of the strawberry acreage in western Washington is now treated with either aldrin or heptachlor.

On November 23, 1954, serious weevil damage was noticed in a strawberry field near Monroe, Washington. This field had been treated with chlordane in 1952, except for one area used for applying soil treatments in previous years. The adult weevils found were not *Brachyrhinus* but *Nemocestes* (*Geoderces*) *incomptus* (Horn), a fairly common weevil in western Washington, and a few *Sciopithes obscurus* Horn, another native weevil. Both species have been mentioned as pests of raspberries (Hanson & Webster 1938). *N. incomptus* is commonly called the raspberry bud weevil. Other workers, at times, have found *N. incomptus* damaging strawberries, but only in a very minor way. The area damaged in this case was small, and no great significance was attached to it except that neither species seemed to be affected by the soil treatment.

On May 3, 1955, *N. incomptus* adults were found in great numbers in this same field. The damaged area had enlarged to include one replication of the area that had previously received soil treatments. The weevils were laying eggs and feeding on the leaves and buds of strawberries, causing considerable damage. Grubs of all sizes were found in the soil, but no counts were made at this

[1] Scientific paper No. 1717 Washington Agricultural Experiment Stations, Project No. 862. Partial cost of publication of this paper was met by the Washington Agricultural Experiment Station. Accepted for publication October 24, 1958.

[2] Associate Entomologist, Northwestern Washington Experiment Station, Mount Vernon.

time because moles had destroyed most of the grubs in some of the plots. The adult population was completely destroyed a few days later by a single application of commercial weevil bait containing sodium fluosilicate and dried apple peelings. Evidently the weevils had laid many eggs before being killed because most of the plants in the area were damaged severely at a later date. Grub counts made (April, 1956) in the one plot the weevils had invaded, indicated that aldrin, dieldrin, heptachlor, chlordane and DDT had no effect on this weevil. Two plots treated with 5 and 7.5 pounds per acre, respectively, of 1% gamma isomer of BHC in April 1952 harbored practically no grubs. During 1955, failure to control *N. incomptus* by treating the soil with aldrin or heptachlor indicated that this weevil might become a major pest of strawberries. In spite of many applications of weevil bait, strawberry plantings adjacent to wooded areas continued to be damaged. A more positive method of control seemed necessary if affected growers were to stay in business.

PROCEDURE.—*Tests in 1956.*—Previous efforts to kill weevil grubs in the soil were discouraging. Most insecticides used as liquids are insoluble in water and must be applied as emulsions or wettable powders. When poured on the soil surface, the water penetrates, but the toxicants are left on the surface. The choice of soil fumigants was limited also, because most of them are highly toxic to plants. Only two of the latter were used: ethylene dibromide (EDB), which previous experience had shown to be fairly safe on strawberries and Nemagon (1,2-dibromo 3 chloro propane of the Shell Chemical Co.), a nemacide which the manufacturers claim is harmless to most plant life. Two water soluble compounds, Dipterex and Vapam (sodium methyldithiocarbamate of the Stauffer Chemical Co.), were also tried.

The first year, plots were small 10-foot sections of single rows, because it was not known whether these materials were toxic to strawberry plants. Twenty-eight plots were located at Lynden,[3] Washington, and 22 at Victoria Heights, Washington, in fields where weevil damage was noticeable. The fumigants were applied with a hand-operated soil injector and the water-soluble compounds were applied with a sprinkling can. The fumigants were applied at 4- and 6-inch depths. In one experiment, where the matted rows were nearly 18 inches wide, injections were made in a single row down the center and fairly close together. These were compared with injections evenly spaced in two rows which gave more even coverage.

The effectiveness of the different treatments was estimated by sifting 1 square foot of soil selected at random in each plot and counting the living larvae present. Check samples from adjacent untreated rows were examined in a similar way. Where larval counts were high in treated plots, indicating no control, examination of all check samples was considered unnecessary. The rates of application given per acre refer to overall coverage. However, because only the area occupied by the matted row need be treated to obtain control, the amount actually required would be approximately one-third of the amounts shown.

TESTS IN 1957.—Test plots were located in four weevil-infested fields in the same two areas as the 1956 trials. The plots in each field were single rows the length of the field with four replicates of each treatment. EDB was compared at four dosages: 0.5 cc., 1.0 cc. and 1.5 cc. per foot of row, and a 50% mixture of 85% EDB plus odorless kerosene at the 1.0-cc. rate. On an acreage basis, treating only the matted row, these applications would equal 1.65 gallons (0.5 cc./ft.), 3.3 gallons (1.0 cc./ft.) and 5 gallons (1.5 cc./ft.), respectively. All except Test 4 were sampled twice on different dates in the same manner as in the 1956 tests.

Test 1.—Victoria Heights, Snohomish County, Washington. Applied May 2, examined May 16–23 and June 7–10. Soil temperature when applied: 53° F. Species involved: *Sciopithes obscurus* only.

Table 1.—Results of treatments to control weevil grubs in strawberry rows, Victoria Heights, Snohomish County, Washington, 1956.

MATERIAL APPLIED	DOSAGE	APPROXIMATE RATE/A.	LIVING INSECTS PER SQ. FT.	
			Treated	Check
	Treated April 11; examined April 24–26			
EDB, 85%	1.0 cc./sq. ft.	11.5 gal.[a]	1	3
	0.7 cc./sq. ft.	8.0 gal.[a]	13	70
	0.4 cc./sq. ft.	4.6 gal.[a]	1	14
	1.0 cc./sq. ft.	11.5 gal.[b]	1	24
	0.7 cc./sq. ft.	8.0 gal.[b]	8	56
	0.4 cc./sq. ft.	4.6 gal.[b]	5	16
Vapam	21.0 cc./plot	12.0 gal.[c]	11	25
	42.0 cc./plot	24.0 gal.[c]	24	1
Dipterex	5.0 gm./plot	25.0 lb.[d]	0	18
	10.0 gm./plot	50.0 lb.[d]	6	32
	20.0 gm./plot	100.0 lb.[d]	6	15
	40.0 gm./plot	200.0 lb.[d]	2	6
	Treated April 26; examined July 12–14			
EDB, 85%	1.7 cc./sq. ft.	20.0 gal.[a]	0	—
	2.6 cc./sq. ft.	30.0 gal.[a]	0	—
	1.7 cc./sq. ft.	20.0 gal.[b]	0	—
	2.6 cc./sq. ft.	30.0 gal.[b]	0	44
Nemagon	0.8 cc./sq. ft.	10.0 gal.[a]	15	6
	0.5 cc./sq. ft.	6.0 gal.[a]	27	0
	1.7 cc./sq. ft.	20.0 gal.[a]	7	—
	0.8 cc./sq. ft.	10.0 gal.[b]	35	18
	0.5 cc./sq. ft.	6.0 gal.[b]	27	—
	1.7 cc./sq. ft.	20.0 gal.[b]	38	—

[a] Applied at 6-inch depth.
[b] Applied at 4-inch depth.
[c] Applied with a sprinkler using 6 gallons of water per plot.
[d] Applied with a sprinkler using 3 gallons of water per plot.

Test 2.—Victoria Heights. Applied May 6, examined May 24–27 and June 24–28. Soil temperature when applied: 54° F. Species involved: *Brachyrhinus sulcatus, B. ovatus,* and *Nemocestes incomptus.*

Test 3.—Victoria Heights. Applied June 3–4, examined June 19–20 and June 21–24. Soil temperature when applied: 59° F. Species involved: *Nemocestes incomptus* only.

Test 4.—Near Lynden, Whatcom County, Washington. Applied October 1, examined November 19–20. Soil tem-

[3] Grateful acknowledgment is extended to Sverre Klingenberg and Pete Snartemo of Victoria Heights and Melvin Cowin of Lynden for the use of parts of their fields in these tests.

perature when applied: 59° F. Species involved: *Nemocestes incomptus* only.

For these tests, a mechanical applicator similar to injection systems used for nematode control (Page & Jenson 1956) was assembled on a small garden tractor. This machine was designed to treat but one row at a time, using a single chisel following an 18-inch plow coulter which cut through the center of the matted row without tearing out the plants. The equipment consisted of a marine gear pump operated by a power take-off, a line strainer, pressure regulator, pressure gauge, shut-off valve and a metering disc at the top of the chisel. Polyethylene tubing was used to connect the various components. The fumigant was pumped directly from a 5-gallon can set in a bracket in the tractor frame. With the tractor set at a constant speed, the flow was regulated by the pressure and the size of the disc.

Table 2.—Results of treatments[a] to control weevil grubs in strawberry rows, Lynden, Whatcom County, Washington, 1956.

Material Applied	Dosage	Approximate Rate/A.	Living Insects per Sq. Ft. Treated	Check
Dipterex, 50%	5.0 gm./plot	25 lb.[b]	33	22
	10.0 gm./plot	50 lb.[b]	24	—
	20.0 gm./plot	100 lb.[b]	39	—
	5.0 gm./plot	25 lb.[c]	79	42
	10.0 gm./plot	50 lb.[c]	43	—
	20.0 gm./plot	100 lb.[c]	3	—
	5.0 gm./plot	25 lb.[d]	20	—
	10.0 gm./plot	50 lb.[d]	5	23
EDB, 85%	0.5 cc./sq. ft.	6 gal.[e]	11	40
	0.8 cc./sq. ft.	10 gal.[e]	0	14
	1.0 cc./sq. ft.	12 gal.[e]	0	—
	0.5 cc./sq. ft.	6 gal.[f]	1	16
	0.8 cc./sq. ft.	10 gal.[f]	0	9
	1.0 cc./sq. ft.	12 gal.[f]	2	25
EDB+kerosene, 1–4	1.0 cc./sq. ft.	12 gal.[e]	1	29
	2.0 cc./sq. ft.	24 gal.[e]	0	45
	4.0 cc./sq. ft.	48 gal.[e]	0	15
	1.0 cc./sq. ft.	12 gal.[f]	1	—
	2.0 cc./sq. ft.	24 gal.[f]	0	—
	4.0 cc./sq. ft.	48 gal.[f]	0	—
Nemagon,	1.0 cc./sq. ft.	12 gal.[e]	11	—
	2.0 cc./sq. ft.	24 gal.[e]	20	19
	5.0 cc./sq. ft.	60 gal.[e]	20	—
	7.0 cc./sq. ft.	96 gal.[e]	20	—
	1.0 cc./sq. ft.	12 gal.[f]	20	—
	2.0 cc./sq. ft.	24 gal.[f]	24	—
	5.0 cc./sq. ft.	60 gal.[f]	19	—
	7.0 cc./sq. ft.	96 gal.[f]	17	—

[a] Treated June 29–July 5; examined August 8–10.
[b] Applied with a sprinkler using 1 gallon of water per plot.
[c] Applied with a sprinkler using 3 gallons of water per plot.
[d] Applied with a sprinkler using 6 gallons of water per plot.
[e] Application staggered.
[f] Application in single line.

Table 3.—Living grubs in plots treated with ethylene dibromide.[a] Victoria Heights and Lynden, Washington, 1957.

Treatment (cc./sq. ft.)	Test 1 Counts 1	Test 1 Counts 2	Test 2 Counts 1	Test 2 Counts 2	Test 3 Counts 1	Test 3 Counts 2	Test 4 Count 1	Average
EDB, 0.5	4.5	3.0	10.8	3.0	8.3	10.3	7.3	6.7
EDB, 1.0	1.0	3.5	5.3	2.5	5.3	3.5	0.0	3.0
EDB, 1.5	0.0	0.8	4.0	6.0	2.0	2.3	0.3	2.2
EDB+Kerosene, 1	1.3	4.8	5.8	4.8	7.5	7.0	7.8	5.5
Check	78.0	70.5	96.3	45.8	42.5	44.5	50.0	61.1

[a] Each figure gives the average number per square foot in four replicates.

DISCUSSION OF RESULTS.—Results of the tests conducted in 1956 are shown in tables 1 and 2. Of the materials used, only Vapam was phytotoxic. Plants in both plots treated with this material died within a few days. Ethylene dibromide apparently stimulated growth, even in plots where few weevil grubs were found. All dosages of EDB gave control; the optimum dose was 1 cc. per square foot. The depth of application made no difference in control, nor did staggering the points of injection to give more even coverage of the area in the row. There was also some indication that EDB could be diluted with kerosene to advantage. Nemagon had little, if any, effect of the grubs of this weevil. Dipterex had some effect in the earlier test when grubs were small, but in a later test only the highest dosages gave any reduction in number of grubs.

All treatments made in 1957 gave good commercial control. The plots treated early in May, when symptoms of insect damage first were noticed, recovered promptly and bore a good crop. Plants in plots treated in June did not recover.

Unlike the common species of strawberry weevil which has a fairly definite life history pattern, *N. incomptus* overwinters equally well as adults and as larvae. In 1956, in the plots at Lynden and at Victoria Heights, the first adults were found on July 11–12. Only about one-third of the grubs had pupated at that time; emergence continued well into November.

It appears that baits are more successful against the adults if applied in the summer before the rainy season begins; consequently, in bait-treated fields, most of the eggs are laid by adults emerging late. Larvae from these eggs over-winter as very small grubs and damage appears about May of the next year. No definite date for fumigating can be set, however. Each grower must watch for symptoms (stunted plants with red leaves) and start treatment when such symptoms appear.

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Notes on Aphids, Psyllids and Whiteflies of Arizona[1]

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Abstract

There are 32 species of aphids (comprising 15 genera), 6 of psyllids (comprising 5 genera) and 8 of whiteflies (comprising 6 genera) listed along with hosts on which, where and when they were collected in Arizona from 1953 to 1958. Occasional notes and comments are included as to their habits and abundance and as to other observations, such as parasitization. The aphid records indicate that *Myzus persicae* (green peach aphid) was the most polyphagous species of the aphids.

To further the knowledge of the insect fauna of Arizona, a list of 32 species of aphids (comprising 15 genera), 6 of psyllids (comprising 5 genera) and 8 of whiteflies (comprising 6 genera) is given below, along with host, locality and date records and occasional notes and comments as to habits, abundance and other observations. The collections from which the list was compiled were made in Arizona during 1953–58 by the writer and associates. The identifications were made, through the courtesy of the Insect Identification and Parasite Introduction Laboratories of the United States Department of Agriculture, by Lousie M. Russell.

Aphids:

Amphorophora sonchi (Oestl.)

"Milkweed?" (probably *Sonchus* sp.): Mesa, IV-5-57 (P. D. Gerhardt), heavy infestation.

Anuraphis helichrysi (Kltb.), leaf curl aphid

Chrysanthemum: Phoenix, IV-16-57 (Ritchie & Zimmer).
Encelia sp.: Tempe, III-30-57.
Phacelia sp.: Tempe, III-30-57.

Anuraphis tulipae (Fonsc.), tulip bulb aphid

Carrot: Mesa, III-19-57 (P. D. Gerhardt)

Aphis fabae Scop., bean aphid

Pittosoporum: Phoenix, III-15-58, severe, some becoming parasitized.

Aphis gossypii Glov., cotton or melon aphid

Cantaloupe: Yuma, IX-30-53 (Don McCain); X-9-53 (H. Czajkowski).
Cauliflower: Phoenix, XI-24-53 (L. A. Hammers).
Cercidium floridum: Phoenix, IV-28-57.
Citrus: Phoenix, III-30-55 (R. L. Dezember) and III-5-57.
Cotton: Buckeye, IV-5-54 (Bill Zimmer); Yuma, IX-8-53 (Don McCain) and IX-30-53.
Encelia farinosa: Tempe, III-30-57, several specimens in association with other aphids.
Grape: Phoenix, IV-16-57 (Ritchie & Zimmer), a sample of 33 specimens received but no report of damage or control.
Lettuce: Yuma, X-29-53 (H. Czajkowski).
Lycium sp: Eloy, III-21-56.
Lupinus sp.: Peralta Canyon (Pinal Co.), along with another aphid.
Leucophyllum Texanum: Chandler, III-7-57 (Herb Brown), heavy infestation.
Malva parviflora: Gila Bend, III-15-55 (Chas. Davis).
Petunia: Phoenix, IV-18-57 (Ann & Mike Sullivan), few specimens in association with many of another aphid.
Pyracantha: Phoenix, III-17-57, several specimens in association with few of another aphid.

Aphis helianthi Mon.

Encelia farinosa: Tempe, III-30-57, several specimens in association with other aphids.
Helianthus sp. (wild sunflower): Phoenix, XI-7-55, numerous alate and apterous adults along with many nymphs on either side of leaves.
Phacelia sp.: Tempe, III-30-57, along with other aphids.
Pittosporum: Phoenix, III-2-55.

Aphis medicaginis Koch, cowpea aphid

Bermuda grass: Roll, V-1-56 (Pete Segulja).
Citrus: Phoenix, III-30-55 (R. L. Dezember).
Cotton: Bowie, IV-14-53 (Clint Allen); Elfrida, V-10-53 (Clint Allen); Eloy, V-13-57 (Skip Hornney), severe in terminals; Maricopa, IV-9-56 (Herb Brown); Pinal County, IV-30-54 (Jack Warren), along with another aphid; Tucson, V-23-55 (Clint Allen), along with another aphid.
Heterotheca subaxillaris: Luke Field (Maricopa Co.), V-26-56.
Olneya tesota: Tempe, V-25-55.
Robinia sp.: Douglas, IV-25-53 (Clint Allen).
Sesbania: Yuma, IX-29-53 (H. Czajkowski).
Tribulus terrestris: Marana, VI-11-54 (Jack Warren).

Aphis nerii Fonsc., oleander and milkweed aphid

Oleander: Phoenix, during winter, usually severe.

Aphis pseudohederae Theob., false ivy aphid

Ivy: Phoenix, II-11-56.

Aphis spiraecola Patch, spirea aphid

Photinia: Phoenix, IV-15-56.
Pyracantha: Phoenix, III-17-56, along with another aphid.
Quince: Phoenix, IV-15-56, severe with some parasitized but, as observed by Miller (1929), no parasites emerged.

Aphis tetrapteralis Ckll.

Larrea tridentata: Tempe, III-27-57.

Brevicoryne brassicae (L.), cabbage aphid

Cabbage: Phoenix, XI-20-53 (L. A. Hammers); I-16-55 (Joe Hartsig), severe on 40-acre field.
Cauliflower: Peoria, III-18-54 (L. A. Hammers).
Capsella bursa-pastoris: Luke Field (Maricopa Co.) III-10-57.
Franseria deltoidea: Tempe, II-17-54.

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Capitophorus sp.

Franseria deltoidea: Phoenix, III-6-54 (Jack Warren).

Chaitophorus populellus G. & P.

Populus Fremontii: Phoenix, III-4-58, abundant on tender foliage.

Chaitophorus viminalis Mon.

Salix sp.: Luke Field, III-14-58 (Dale Berry), on new growth.

Hyalopterus atriplicis (L.)

Cotton: Luke Field, V-29-55, 1 specimen, along with another aphid.

Hyalopterus arundinis (F.), mealy plum aphid

Carrizo (bamboo): Yuma, X-29-53 (Don McCain).

Hysteroneura setariae (Thos.), rusty plum aphid

Apricot: Phoenix, XI-8-57, heavy infestation.

Corn: Gilbert, IX-21-56.

Grain sorghum: Peoria, IX-6-55 (Ben Fehrman), and many other fields in Maricopa County, all with periphery more heavily infested than interior; one in which the aphid was highly parasitized by *Aphelinus nigritus* How. (det. B. D. Burks).

Johnson grass: Gilbert, IX-21-56, abundant on stunted plants under which much honeydew had accumulated.

Macrosiphoniella sanborni (Gill.), chrysanthemum aphid

Chrysanthemum: Phoenix, XI-25-53 (Joe Hartsig) and IV-16-57 (Ritchie & Zimmer).

Macrosiphum ambrosiae (Thos.)

Ambrosia artemisiifolia: Phoenix, IV-28-57; Yuma, IV-5-56 (Stu Evans).

Lettuce: Aguila, III-17-58 (Pace & Zimmer).

Macrosiphum barri Essig

Lactuca: Mesa, IV-5-57 (P. D. Gerhardt), severe.

Macrosiphum granarium (Kirby), English grain aphid

Barley: Avondale, III-14-55 (Bill Zimmer); Bowie, V-10-55, severe in some fields; Pinal County, IV-2-56 (Herb Brown), along with another aphid; Scottsdale, II-27-56 (Fred Corbus).

Wheat: Casa Grande, IV-14-55 (Daley & Lofgreen), severe; Eloy, IV-16-57 (Skip Hornney); Marana, IV-16-56 (Ken Brinkmeyer), with preference for wheat to barley strongly indicated; Peoria, IV-18-58 (Dale Berry); Wellton, III-15-56 (Pete Segulja).

Macrosiphum pisi (Harris), pea aphid

Alfalfa: Perryville, IV-1-55; Yuma, II-10-54 (Don McCain).

Potato: Queen Creek, IV-6-56 (I. L. McMahon), alate and apterous forms.

Observed in many other instances at various places, principally on legumes.

Macrosiphum solanifolii (Ashm.), potato aphid

Cantaloupe: Yuma, IV-16-56 (L. A. Hammers), along with another aphid.

Citrus: Phoenix, III-5-57, numerous.

Cotton, Peoria, IV-18-58 (Dale Berry); Waddell, IV-26-56 (Bill Zimmer), numerous.

Lettuce: Kansas Settlement (Cochise Co.), V-14-58 (John Merrick), an apterous, summer vivipara.

Petunia: Phoenix, IV-18-57 (Ann & Mike Sullivan), numerous, along with another aphid; Tempe, IV-22-56, severe.

Pima cotton: Phoenix, V-26-56 (Bill Zimmer), along with another aphid.

Rose: Phoenix, IV-6-57 (Connie & George Holt) and IV-22-58.

Monellia costalis (Fitch), black-margined aphid

Lettuce: Yuma, X-26-53 (H. Czajkowski), may have flown or been blown from infested pecan trees, nearby, but there were apterous forms present and there seemed to have been reproduction and feeding on the lettuce.

Pecan: Phoenix, X-10-53, severe every year at Phoenix and elsewhere in the state.

Myzus persicae (Sulz.), green peach aphid

Actinea odorata: Maricopa, IV-9-56; Stanfield, IV-17-58 (George Wene).

Barley: Chandler, III-6-57 (R. L. Dezember), in association with another aphid.

Bermuda grass: Roll, V-1-56 (Pete Segulja), in association with another aphid.

Cantaloupe: Somerton, III-14-57 (Don McCain), along with another aphid; Yuma, IV-16-56 (L. A. Hammers), along with 2 other aphids.

Capsella bursa-pastoris: Luke Field, III-10-57, along with another aphid.

Cerdidium floridum: Phoenix, IV-28-57, along with another aphid.

Citrus: Phoenix, III-5-57, abundant.

Cotton: Coolidge, IV-22-54 (Art Lofgreen), abundant in one field; Maricopa County, IV-26-56 (Bill Zimmer), along with another aphid; Mesa, IV-8-57 (R. L. Dezember), all alate; Pinal County, IV-30-54 (Jack Warren), along with another aphid; Tucson, V-23-55 (Clint Allen), along with another aphid; Yuma, V-18-55.

Cucumber: Yuma, III-13-57, in epidemic numbers, probably resulting from an immigration of alates.

Encelia farinosa: Peralta Canyon (Pinal Co.), III-31-57; Tempe, III-25- and 30-57.

Grain sorghum: Eloy, IV-16-57 (Skip Hornney).

Grape fruit: Phoenix, III-30-55 (R. L. Dezember), along with another aphid.

Heliotrope: Roll, III-15-57.

Johnson grass: Phoenix, III-3-54.

Larrea tridentata: Tempe, III-27-57, along with 3 other aphids.

Lettuce: Chandler, III-21-57 (R. L. Dezember); Eloy, 2-26-56 (Skip Hornney); Harquahala, III-21- and 28-57 (Chas. Davis); Phoenix, II-18-54 (L. A. Hammers); II-24-55, abundant, along with another aphid and III-18-57, along with another aphid; Yuma, X-29-53 and I-4-54 (H. Czajkowski).

Lupinus sp.: Peralta Canyon (Pinal Co.), III-31-57; along with another aphid.

Malva parviflora: Gila Bend, III-15-55 (Chas. Davis); Phoenix, I-6-54; III-4-58, none parasitized; Somerton, III-13-57 (Don McCain).
Milo maize: Eloy, IV-16-57 (Skip Hornney), 1 specimen, along with other aphids.
Mustard: Marana, IV-30-56, along with 2 other aphids; Picacho, X-28-53 (Jack Warren).
"Myrtle vine": Wellton, III-2-55 (L. L. Brimhall).
Nightshade: Peralta Canyon (Pinal Co.), III-31-57.
Phacelia sp.: Tempe, III-30-57, among which were males and viviparous females, along with 2 other aphids.
Potato: Harquahala, III-28-57 (Chas. Davis); Maricopa, III-30-56 and III-21-58 (Herb Brown); Queen Creek, IV-23-54; Somerton, III-13-57 (Don McCain); Stanfield, III-7-57 (Herb Brown): a serious and persistent pest of potato.
Radish: Phoenix, IV-5-55, along with another aphid.
Ranunculus: Phoenix, II-28-55 (R. L. Dezember).
Rumex sp.: Apache Junction, III-10-57 (R. L. Dezember).
Sambucus sp.: Phoenix, V-26-55.
Sisymbrium sp.: Mesa, III-1-54, along with another aphid; Peoria, II-18-54 (L. A. Hammers); Phoenix, I-11- and II-3-54.
Sphaeralcea sp.: Casa Grande, I-23-56 (R. L. Dexember), abundant on a colony of plants.
Solanaceous ornamental shrub: Yuma, IV-16-56 and III-14-57, abundant.
Solanum elacagnifolium: Queen Creek, V-23-55 (Art Lofgreen).
Squash: Yuma, III-13-57 (Don McCain), many alate and some apterous specimens.
Sugar beet: Mesa, V-12-55; Tolleson, I-26-58; a common pest of this crop wherever grown in Arizona.
Verbesina enceloides: Roll, III-15-57.
Wheat: Peoria, IV-18-58 (Dale Berry).

Rhopalosiphum fitchii (Sand.), apple grain aphid

Barley: Scottsdale, April, 1957, (Corbus & Dezember).
Oats: Phoenix, VI-18-58 (Dale Berry); Somerton, March, 1957 (Don McCain), severe in some fields.

Rhopalosiphum maidis (Fitch), corn leaf aphid

Barley: Avondale, III-14-55 (Bill Zimmer); Beardsley' XIII-30-54 (Bill Zimmer); Chandler, III-6-57 (R. L. Dezember); Coolidge, 1-10-54; Eloy, III-25-56 (Skip Hornney); Gilbert, III-1-57 (R. L. Dezember); Mesa, III-21-56 (P. D. Gerhardt); Phoenix, 1-28-55 and III-10-57 (Dale Berry); III-28-57 (Bill Zimmer); Roll, III-15-57 (Pete Segulja), heavy infestation at top of plants, ready to head; Tolleson, XII-30-54; Tucson, V-23-55 (Clint Allen).
Bermuda grass: Yuma, V-20-55 (Don McCain).
Cantaloupe: Somerton, II-14-57 (Don McCain); Yuma, IV-16-56 (L. A. Hammers).
Cauliflower: Phoenix, XI-24-53.
Cucumber: Yuma, III-13-57 (Don McCain)
Grain sorghum: Eloy, IV-16-57 (Skip Hornney); Gilbert, IX-15-53; Mesa, VIII-9-53; Phoenix, X-14-55; Yuma, IX-29-54.
Johnson grass: Phoenix, IX-23-54; Mesa, IV-8-57.
Lettuce: Phoenix, IX-23-54; Yuma, Oct., 1953.
Milo maize, Eloy, IV-14-57 (Skip Hornney), along with 2 other aphids.
Mustard: Marana, IV-30-56.
Sphaeralcea sp.: Scottsdale, III-3-57.
Wheat: Maricopa, I-20-56 (Herb Brown); Somerton, March, 1957 (Don McCain), along with another aphid.

Rhopalosiphum nymphaeae (L.), water-lily aphid

Ranunculus: Phoenix, II-28-55 (R. L. Dezember), along with another aphid.

Rhopalosiphum pseudobrassicae (Davis), turnip aphid

Sisymbrium sp.: Mesa, III-1-54, along with another aphid in greater numbers.

Therioaphis maculata (Buckton), spotted alfalfa aphid

Alfalfa: Buckeye, VI-12-54 (Chas. Davis), severe; Yuma, May, 1954 (Don McCain), severe; Southern Arizona, May and June, 1954) (Tuttle & Butler 1954), spreading rapidly and becoming a severe pest of alfalfa over the entire state; greatly subsiding by 1957, such probably resulting from natural control among which was included an entomogenous fungus, *Entomopthora*, (det. C. G. Thompson) at Buckeye, November, 1955 (Chas. Davis); and indications that an introduced parasite, *Trioxys utilis* Muesebeck (det. O. L. Barnes), was becoming well established and effective at Buckeye, spring, 1958 (Berry & Zimmer).

Toxoptera graminum (Rond.), greenbug

Barley: Pinal County, IV-2-56 (Herb Brown), very light infestation.
Oats: Phoenix, VI-10-54 (Stu Evans), along with another aphid.
Milo maize (2–3 inches high): Eloy, IV-16-57 (Skip Hornney), mostly on crown but some definitely on roots and a few on foliage, sample for identification comprising 20 specimens, along with 7 of two other aphids.

In keeping with the records of Patch (1938) in which *Myzus persicae* was recorded from 315 species of 65 of 155 phylogenetic groups of plants; and of Palmer (1952) in which collections were listed from 67 species of various families, the records above indicate that it was highly polyphagous; more so than any other aphid included in the list.

PSYLLIDS:

Aphalara gutierreziae Klyver

Aplopappus sp.: Tempe, II-17- and 24-54.

Aphalara suaedae Crawf.

Suaeda torreyana: Buckeye, X-23 and XI-20-53 (Bill Zimmer), many nymphs and adults; Maricopa, VII-24- and 28-56: probably of no economic importance except to perpetuate and further natural enemies of similar pests on economic crops, in a manner similar to that of *Trichogramma minutum* Riley transferring from amaranth leaf tier on amaranth to celery leaf tier on celery as observed by Ball *et al.* (1935).

Aphalaroida sp.

Acacia sp.: Casa Grande, V-21-56.
Larrea tridentata: Phoenix, III-5-54.

Leuronota maculata (Crawf.)

Cotton: Tucson, IV-3-56 (Clint Allen), only 1 specimen.
Celtis pallida: Tempe, IV-3-56 (E. R. Blakley), causing tender leaves to curl.

Paratrioza cockerelli (Sulc), potato psyllid

Baccharis sp.: Avondale, I-22-54; Pinal County, I-16-54; Tempe, I-18-54: all adults probably hibernating.
Franseria deltoidea: Tempe, II-16-54, adults probably resting or accidental.
Lycium spp.: Phoenix area, II-3- and 4-57, eggs and adults but no nymphs probably because of below-normal temperature with frost some mornings for the previous two weeks; Pinal County, III-3-54 (Jack Warren); Tempe, II-13-55, III-6- and 10-55, eggs, nymphs and adults; Scottsdale, III-11-55, eggs and nymphs.
Potato: Where grown throughout the state on spring crop, eggs having been found by March 21 (Ben Fehrman).

Trioza collaris Crawf.

Baccharis sp.: Avondale, I-22-54, 1 adult.

WHITEFLIES:

Aleurocybotus sp.

Bermuda grass: Yuma, IX-30-53 (Don McCain), severe.
Grain sorghum: Pinal County, X-19-53 (Dick Daley); Yuma, IX-29-53 (Hank Czajkowski), immature stages.

Aleyrodes sp.

Baccharis sp.: Luke Field, VI-12-55, heavy infestation of eggs, larvae, pupae and adults on some leaves.

Bemisia sp.

Dodonea sp.: Mesa, III-5-54 (Lemac Hopkins), 1 specimen.

Tetraleurodes acaciae (Q.)

Olneya tesota: Tempe, X-4-54.

Tetraleurodes mori (Q.)

Fraxinus sp.: Phoenix, V-21-55.

Tetralicia sp.

Ambrosia artemisiifolia: Luke Field, V-26-55.
Mesquite: Mesa, XII-4-53, larvae and pupae, black.

Trialeurodes abutilonea (Hald.)

Ambrosia artemisiifolia: Luke Field, V-26-57.
Cotton: Pinal County, V-15-54 (Jack Warren), severe.
Franseria ambrosiodes: Cave Creek, XI-6-57.
Potato: Pinal County, V-7-54.
Sphaeralcea sp.: Phoenix, X-7-53 (Bill Ritchie).

Trialeurodes floridensis (Q.)

Dodonea sp.: Mesa, III-5-54 (Lemac Hopkins), many specimens.

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Laboratory Studies of Residual Contact Effect of 28 Insecticides on Japanese Beetle Adults[1]

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ABSTRACT

Twenty-eight organic insecticides were tested against Japanese beetle adults, *Popillia japonica* Newm. Different deposit rates on glass surfaces were aged for up to 35 days. The effectiveness of these deposits was evaluated against Japanese beetle adults during the aging period. Of the materials tested, Guthion and Sevin (N-methyl-1-naphthyl carbamate) proved to be the most effective both in toxicity and length of residual life. Phosdrin and RE-4355 [*O,O*-Dimethyl-*O*-(1,2-dibromo-2,2 dichloroethyl) phosphate] were the most rapid in action.

The Japanese beetle, *Popillia japonica* Newm., although present in some areas of North Carolina, is not generally considered a major crop pest. During the summer of 1957, however, extremely heavy adult populations seriously damaged soybeans, sweet corn and apple trees in some sections. A review of the literature revealed that nearly all recent control work has been directed against the larvae with little information on the control of the adults.

DDT and methoxychlor have long been the recommended insecticides for the control of adult Japanese beetles, but the control has not always been satisfactory. In an attempt to find more effective materials Langford *et al.* (1951) tested chlordane, TDE, toxaphene, dieldrin, aldrin, parathion, and lindane and concluded from laboratory and field studies that these materials were as

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effective as DDT or methoxychlor. Siegler (1952) stated that parathion was the most effective material when freshly applied sprays were tested, but that it had no residual action. In 1953, Langford *et al.* used Dilan, EPN, malathion, Metacide, schradan, NPD, demeton, DDT, lindane, and methoxychlor in laboratory tests and found that all these materials were toxic to the adult beetle. The results with schradan and demeton, however, indicated that they were not effective as systemic insecticides but only when applied directly on the insect.

In none of the tests reported above was the actual deposit of insecticide given. In order to obtain an evaluation of the afore-mentioned materials in relation to the newer insecticides, laboratory tests were conducted using adult beetles on known deposits of insecticides. Twenty-eight organic insecticides were tested.

METHODS.—Adult Japanese beetles were collected from sweet corn and soybeans near Faison, N. C., during the first half of July 1957 and from smartweed near Hendersonville, N. C., during the second half of the month. Adult beetles were held in large screen cages in a laboratory at 78° F. and 75% relative humidity until used. Food consisted of soybean leaves supplied fresh daily.

All insecticides were used as emulsifiable concentrates diluted with water and applied to the bottom half of petri dishes by means of a calibrated spray tower. The deposit was calculated in micrograms of insecticide per square centimeter, 10.9 μg./cm.^{2} being equivalent to 1 pound per acre of surface. The emulsifiable concentrates were made of 20 grams of technical insecticide, 10 grams of emulsifier (Atlox 1045A), and sufficient xylene to make 100 ml. In those cases where the technical material was not soluble in xylene, acetone was used.

The tests were of two types; those evaluating the residual toxicity of the insecticide films, and those used to determine the LD-50 values of freshly applied deposits. The residual toxicity tests were conducted by exposing adult beetles to deposits of insecticides on glass petri dishes that had aged for various lengths of time. The LD-50 values were obtained by exposing adult beetles to freshly treated surfaces; however, LD-50 values were only determined for those materials that show superior residual action.

The initial deposit in the residual toxicity tests was 10 micrograms per square centimeter. The deposit rate was then continually halved until practically no mortality resulted. As soon as the deposit had dried, adult beetles were confined in the dishes for a 24-hour period. Mortality counts were then made and the dishes uncovered until the next 24-hour test period. The age of the residue was recorded at the initiation of each test period. Ten insects were used per concentration on the residual tests. The LD-50 values were determined from four deposit rates of each material using 200 insects for each rate. The deposits used were as follows: TDE, DDT, and methoxychlor, each 1.2, 0.6, 0.3, and 0.15 microgram per cm.^{2}; endrin, 0.6, 0.3, 0.15, and 0.07 microgram per cm.^{2}; Sevin and Guthion, each 0.076, 0.034, 0.019, and 0.009 microgram per cm.^{2} Test procedures were the same as for the residual tests.

Check insects were included with each day's testing, using a ratio of one check dish to each 10 treated dishes. The average check mortality for the entire test was 10.6%.

An insect was considered dead when it failed to show any signs of movement after being prodded with a needle. Insects that were moving but unable to stand upright or crawl were considered moribund. Practically all moribund beetles were dead when held for an additional 24-hour period.

Residues were aged, and treated insects were held in a constant temperature room at 75° F. and 70% relative humidity.

RESULTS AND DISCUSSION.—The results are shown in tables 1 and 2.

Of the 28 materials tested, only six had residues that remained toxic for more than 9 days. These residues were

Table 1.—Residual effectiveness of 28 insecticides to adult Japanese beetles.

Residue Insecticide	Deposit in μG./Cm.^{2}	Per Cent Kill[a] Age of Deposit in Days 0	4	9
Aldrin	10	100	(100)	0
	5	100	30	0
	2.5	80	0	0
	1.25	10	20	0
Bayer 19639	10	100	60	0
	5	100	(100)	0
	2.5	100	(100)	0
	1.2	90	10	0
	0.6	80	0	0
	0.3	0	0	0
Chlorthion	10	100	0	0
	2.5	100	0	0
	1.25	(100)	10	20
	0.6	70	0	0
	0.3	60	30	10
	0.15	70	0	0
	0.07	20	10	0
Chlordane	10	100	(50)	10
	5	(100)	0	0
	2.5	70	0	0
	1.2	100	0	0
	0.6	70	0	0
	0.3	50	0	0
DDT	10	90	(100)	(100)
	2.5	(100)	(100)	(100)
	1.25	(100)	90	30
	0.6	100	50	0
	0.3	(100)	50	0
	0.15	30	30	0
	0.07	0	0	0
DDVP	10	100	0	0
	2.5	(100)	0	0
	1.2	10	0	0
	0.6	30	0	0
Demeton	10	100	0	0
	5	100	10	0
	2.5	(90)	0	0
	1.2	30	40	0
	0.6	40	0	0
Diazinon	10	100	100	90
	5	100	100	0

[a] Figure in parenthesis is per cent dead plus moribund.

Table 1.—(*Continued*)

Residue Insecticide	Deposit in μg./Cm.²	Per Cent Kill[a] Age of Deposit in Days 0	4	9
	2.5	100	100	0
	1.2	60	0	0
Dieldrin	10	100	(100)	(100)
	5	(100)	(100)	0
	2.5	(100)	0	0
Dipterex	10	100	(100)	0
	2.5	100	10	10
	1.2	100	10	10
	0.6	90	30	20
	0.3	60	10	20
	0.15	60	20	0
Dow ET-57[b]	10	100	0	0
	5	100	0	0
	2.5	100	10	0
	1.2	20	30	0
	0.6	10	10	20
	0.3	0	0	0
Endrin	10	100	(100)	(100)
	5	(100)	(100)	(20)
	2.5	(100)	10	(20)
	1.2	100	20	0
	0.6	100	30	10
	0.3	(100)	40	10
	0.15	20	0	0
	0.07	0	0	0
Guthion	10	100	100	100
	2.5	100	100	100
	0.6	100	(100)	100
	0.3	100	20	40
	0.15	100	30	10
	0.07	80	20	20
	0.037	80	30	20
	0.019	0	0	0
Heptachlor	10	100	(90)	10
	5	(100)	10	0
	2.5	(100)	10	0
	1.2	(100)	20	0
	0.6	(100)	20	0
Lindane	10	100	40	0
	5	100	(70)	0
	2.5	100	(90)	0
	1.2	100	40	40
	0.6	(100)	30	20
	0.3	(100)	10	10
	0.15	60	30	0
	0.07	20	10	10
	0.037	10	10	0
Malathion	10	100	100	20
	5	100	40	0
	2.5	100	(100)	10
	1.2	100	10	0
	0.6	100	20	20
	0.15	(90)	0	0
	0.07	(100)	0	0
	0.037	0	0	0
Methoxychlor	10	100	(100)	(100)
	5	100	(100)	(100)
	2.5	(100)	(60)	(50)

[b] Sampled as Dow ET-14; also called Korlan.

Table 1.—(*Continued*)

Residue Insecticide	Deposit in μg./Cm.²	Per Cent Kill[a] Age of Deposit in Days 0	4	9
	1.2	(100)	(100)	(100)
	0.6	(100)	(90)	(70)
	0.3	(100)	60	0
	0.15	(100)	0	0
	0.07	0	0	0
	0.03	0	0	0
Parathion	10	100	100	0
	5	100	100	(90)
	2.5	(100)	100	20
	1.2	100	0	0
	0.3	60	0	0
	0.15	30	0	0
	0.07	50	0	0
	0.03	10	10	0
	0.019	0	0	0
Perthane	10	100	10	0
	5	100	0	0
	2.5	(100)	0	0
	1.2	20	0	0
	0.6	10	0	0
Phosdrin	10	100	100	0
	5	100	100	0
	2.5	100	0	0
	1.2	100	10	0
	0.6	100	10	0
	0.3	100	20	0
	0.15	100	30	0
	0.03	10	0	0
	0.019	30	0	0
	0.009	0	0	0
RE-4355	10	100	20	20
	2.5	100	0	0
	1.2	100	10	0
	0.6	60	10	0
	0.3	20	20	10
	0.15	40	0	0
	0.07	10	0	0
Rotenone	10	100	10	0
	5	100	0	0
	2.5	(80)	0	0
	1.2	100	30	20
	0.6	40	20	0
	0.3	20	0	0
	0.15	20	0	0
Sevin	10	100	100	100
	1.2	100	100	100
	0.6	100	30	(100)
	0.3	100	70	30
	0.15	100	20	20
	0.07	100	0	0
	0.037	50	10	40
	0.019	30	0	0
	0.009	50	20	20
	0.0045	50	10	30
TDE	10	100	(100)	(100)
	2.5	(100)	(80)	(90)
	1.2	(80)	0	0
	0.6	(100)	20	20
	0.3	70	20	10
	0.15	30	20	0
	0.07	10	0	0

(*Continued on page 12*)

Table 1.—(*Continued*)

Residue Insecticide	Deposit in μg./Cm.²	Per Cent Kill[a] Age of Deposit in Days 0	4	9
Thimet	10	100	10	0
	2.5	100	0	0
	1.2	50	0	0
	0.6	50	0	0
	0.3	40	0	0
	0.15	30	0	0
Thiodan	10	100	(90)	0
	2.5	(100)	(100)	0
	1.2	100	10	0
	0.6	(100)	0	0
	0.3	20	0	0
	0.15	20	10	0
	0.07	50	0	0
Toxaphene	10	100	100	0
	2.5	(70)	(100)	0
	1.2	70	0	0
	0.6	40	0	0
Trithion	10	100	10	0
	5	100	(90)	0
	2.5	(100)	0	0
	1.2	100	0	0
	0.6	(100)	0	0
	0.3	40	0	0
	0.15	0	0	0

again tested at 18 and 35 days of age with the results shown in table 2. LD-50 values were obtained for these six materials using fresh residues on glass surfaces (table 3). Sevin and Guthion were the most effective with endrin a close third. DDT, TDE, and methoxychlor were much less effective when only dead beetles were considered; however, if moribund and dead beetles were grouped together, these differences diminished. Methoxychlor in particular, although it had the highest LD-50, was unique in its speed of action. Beetles in the methoxychlor treatment were affected within 1 hour after exposure, and although the kill was low at 24 hours, the beetles were unable to crawl or stand upright.

Sevin and Guthion, while slower in action than methoxychlor, gave positive kills within 4 hours, and at much lower dosage rates. TDE, DDT, and endrin were much slower in action, requiring 10 to 20 hours to have effect on the insects. Furthermore, each of these three materials had a much shorter residual life than Sevin, Guthion, or methoxychlor.

Phosdrin and RE-4355 were extremely rapid in killing action, giving 100% kills within 5 minutes on a freshly treated surface. Both of these materials, however, had a very short residual life, being totally ineffective after 24 hours except at the highest concentration.

Table 2.—Residual effectiveness of six insecticides to adult Japanese beetles at 18 and 35 days.

Residue Insecticide	Deposit in μg./Cm.²	Per Cent Kill[a] Age of Deposit in Days 18	35
DDT	10	(70)	(20)
	5	40	
	2.5	0	
Endrin	10	(30)	
	5	0	
	2.5	0	
Guthion	10	100	100
	5	100	100
	2.5	100	100
	1.25		80
	.06		10
Methoxychlor	10	(100)	(90)
	5	(100)	(100)
	2.5	(100)	(30)
Sevin	10	100	100
	5	100	100
	2.5	100	100
TDE	10	(90)	

[a] Figure in parenthesis is per cent dead plus moribund.

Table 3.—LD-50 values for six insecticides used in surface contact tests against adult Japanese beetles.

Material	Micrograms/Cm.²	LMD-50[a] Micrograms/Cm.²
Sevin	0.042	0.035
Guthion	0.050	0.042
Endrin	0.11	0.07
DDT	0.25	0.09
TDE	1.00	0.30
Methoxychlor	1.60	0.21

[a] LMD-50 includes dead plus moribund beetles.

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Spot Treatments with Malathion Dust for Control of the Northern Fowl Mite on Hens in Individual Wire Cages[1]

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Abstract

Effective control of the northern fowl mite, *Ornithonyssus sylviarum* (C. & F.), by means of spot treatments with 4% malathion dust was demonstrated on three southern California chicken ranches, each with a history of chronic infestation. Infested hens were detected by looking for mites on the eggs each day as they were gathered. Each week, dust was applied only to the infested cage and the three adjacent cages on both sides of the infested cage in the same row. The amount of insecticide needed and the time required for application were markedly reduced from that of previous control programs in which all the cages were treated at each application.

The northern fowl mite, *Ornithonyssus sylviarum* (C. & F.) is a common and recurrent pest of chickens housed in individual-type wire mesh cages (for 1 or 2 hens per compartment) in southern California.

The farmer generally notices mites on eggs here and there throughout a cage plant. At this time infestation of individual hens may vary from light to heavy. Occurrence of the mites in separated spots, high labor costs, and limited time lead many poultrymen to put off treating; inevitably, this is false economy. On the other hand, some operators either treat on a regular schedule, even if no mites are found, or treat the complete plant as soon as a few mites are noticed. Therefore, it seemed desirable to evaluate the efficiency of a program of spot treatments, only, of infested hens as indicated by the appearance of mites on the eggs.

Materials and Methods.—Vincent *et al.* (1954) reported high efficiency of malathion dust against the northern fowl mite when applied by hand to individual chickens or to the litter on the floor of the hen house. The pesticide of choice for tests of the efficacy of a spot-treatment program was, therefore, malathion.

Conditions and methods of handling poultry vary slightly from one ranch to another. For these studies three ranches (A, B, and C) near Riverside were selected. Ranch A had 364 hens, Ranch B had 512, and Ranch C had 3600. At ranches A and B there were two hens in each cage compartment, and at Ranch C a single hen per unit. Each ranch had a history of chronic infestation by the northern fowl mite; repeated applications to all cages were being used for the control of this pest, and the operators were not satisfied with the results.

The first step was detection of mites as soon as possible after the [hen] became infested; this could be done efficiently by examining the eggs for mites as they were gathered each day. (At all three ranches eggs were collected twice daily.) Cages with two hens, each laying at a 75% rate, were subject to a check for mites approximately six times a week per hen. When mites were found on the eggs the cage was marked at the front with a quill feather.

Applications were made at intervals of a week, only the spot-location marked with a feather being treated with 4% malathion dust. Following application of the spot treatment of malathion, the cages were marked by a colored clothespin as a guide to post-treatment examinations. Initially, at Ranch A, only the single infested cage was dusted. As the work progressed, it appeared necessary to treat the hens in cages adjacent to the one in which the mites were found. Thereafter, the three adjacent cages on both sides of the infested cage in the same row were included in the spot treatment. This procedure was employed also on Ranches B and C throughout the test.

At Ranch A, hand-shaker can was used to apply the 4% malathion dust. The hen was caught and held against the top of the cage while the dust was applied to the vent and fluff areas. At Ranches B and C, a hand-operated bellow-type duster of 1-pound capacity was used. A modified nozzle, 3 feet long and curved upward at the end, was attached to the duster. With this device, the hens were treated from below, two puffs being directed to the vent and fluff areas of each hen. The dust was delivered with force very close to these areas and in such a manner as to drive it directly to the skin. This was very important to effective control. Application with the bellows-type duster was preferred. By this means the marked infested cage plus the three on each side of it could be treated quickly and effectively, often in a minute or less, without disturbing the hens by handling them.

Discussion of Results.—The spot-treatment applications required are shown in table 1 by weeks, with

Table 1.—Application intervals and percentages of flock in individual cages dusted with 4% malathion during spot-treatment programs for the control of the northern fowl mite. Riverside, California. 1957–58.

Ranch A			Ranch B			Ranch C		
Week	Hens Treated	Per Cent of Flock	Week	Hens Treated	Per Cent of Flock	Week	Hens Treated	Per Cent of Flock
1	78	21.4	1	42	8.2	1	33	0.9
2	12	3.3	7	1	0.2	2	15	0.4
3	18	4.9	8	1	0.2	3	17	0.5
4	2	0.5	9	10	1.9	4	14	0.4
5	8	2.2	10	1	0.2	6	38	1.0
6	2	0.5	11	6	1.2	7	6	0.2
7	5	1.4	12	3	0.6	8	3	0.1
8	9	2.5	13	1	0.2	9	10	0.3
9	3	0.8	14	3	0.6	10	3	0.1
10	8	2.2	15	3	0.6	11	3	0.1
11	6	1.6	16	2	0.4	12	2	0.1
15	1	0.3	19	1	0.2	13	9	0.2
17	17	4.7	20	1	0.2	14	10	0.3
19	1	0.3	23	1	0.2	16	31	0.9
20	2	0.5	25	5	1.0	17	2	0.1
23	1	0.3	27	2	0.4	18	8	0.2
31	3	0.8	29	4	0.8	19	25	0.7
33	1	0.3	31	5	1.0	20	22	0.6
34	3	0.8	33	4	0.8	21	1	0.0+
43	1	0.3	41	6	1.2	22	11	0.3
48	3	0.8	45	1	0.2	23	14	0.4
50	3	0.8	49	3	0.6	24	6	0.2
51	3	0.8	52	3	0.6	25	9	0.2
52	0	0.0				26	1	0.0+
						28	9	0.2
						29	10	0.3
						30	15	0.4
						33	16	0.4
						40	3	0.1
						44	3	0.1
						45	10	0.3
						46	6	0.2
						52	0	0.0
Average per week	1.00			0.41				0.19

[1] Paper No. 1054, University of California Citrus Experiment Station, Riverside, California. Accepted for publication July 14, 1958.

the number of hens and the percentage of the flock treated at each application. Not only was the percentage of hens infested higher during the first week of the program, but the hens infested carried a relatively heavy infestation of northern fowl mites. After the program of spot treating was under way, inspection showed that populations of northern fowl mites were relatively light on the infested hens found. The vigilance of the operators in daily egg inspections followed by prompt spot treatments not only decreased the percentage of hens infested but controlled the mites before the hens suffered serious stress from the infestation.

Only a single spot-treatment application was required for any given cage; in no instance was it necessary to retreat a cage during the year of the experimental programs. Information is incomplete on the manner in which northern fowl mites arrive at a poultry ranch and are distributed within its premises. No doubt some mites spread from unnoticed infested hens during the program of spot treatments. On the other hand, it is likely that northern fowl mites were brought into the ranches from the outside from time to time, perhaps by some agent or carrier. The English sparrow, *Passer domesticus domesticus* (Linnaeus), is commonly suspected; observations of the populations of English sparrows showed that the number was low at ranches A and B but was high at Ranch C.

In any given week during the course of the spot treatments, the number of infested cages remained relatively low. Although there were periods when treatment was required each week and applications were made in approximately half of the weeks, at the end of the year of the experimental program mites had been found in a total of only 30.8% of the cages at Ranch A, 13.1% at Ranch B, and 9.2% at Ranch C, exclusive of the first week. The amount of insecticide and labor used in the spot treatments for the year was considerably less than that of a single application to the entire ranch. Previously, with complete treatment of all cages at each application, infestations of northern fowl mite had recurred several times a year.

Since the spot treatments each week could be given in a short time, or even piecemeal, the labor could be fitted into the regular work schedule and taken care of without extra help or special arrangements.

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The Effectiveness of Dieldrin against the Rice Water Weevil[1]

W. MATHIS and H. F. SCHOOF[2]

ABSTRACT

In studies on the control of rice field mosquitoes in Bolivar County, Mississippi, in 1952–53, observations indicated that the dieldrin treatments employed were highly effective against the larvae of rice water weevil, *Lissorhoptrus oryzophilus* Ksl. Airplane applications of dieldrin emulsion at rates of 0.5 or 1.0 pound per acre almost completely eliminated weevil infestations in rice fields (48 to 100 acres) during the entire 9- to 10-week test period. Small plot tests with dieldrin as an emulsion, impregnated pellets, or as a seed treatment, also were highly effective.

In 1952–53, studies were conducted in Bolivar County, Mississippi, to determine effective measures for control of *Psorophora* breeding in rice fields (Mathis *et al.* 1954). During these tests it became apparent that chemical treatments for mosquito control also were of value against rice water weevil infestations. Since rice field irrigation practices for weevil reduction favor heavy production of *Psorophora confinnis* (L.-Arr.) and *P. discolor*, (Coq.), a chemical measure which will control both mosquito and weevil infestations is highly advantageous, not only from the stand-point of its dual efficacy but also from a public acceptance viewpoint. Consequently, in the various mosquito control tests, parallel evaluations also were made on rice water weevil densities.

The rice water weevil (*Lissorhoptrus oryzophilus* Ksl.) first attracted the attention of rice farmers in areas along the Savannah River in Georgia and South Carolina. In the report of the United States Commissioner of Agriculture for 1881 and 1882 (Tucker 1912), Dr. L. O. Howard recommended draining the fields and then allowing them to dry sufficiently to cause the death of the weevil larvae. Although adult feeding upon the leaf blades may occasionally injure young rice, the larvae, which attack the water-submerged roots, cause the greatest damage (Isley & Schwart 1932). The effectiveness of the recommended irrigation measure depends upon the degree to which the field dries. Detrimental effect to the plants also may occur when the fields thoroughly dry, particularly if the roots are heavily infested with weevil larvae (Tucker 1912). Other objections to the drainage practice are water wastage, the difficulty encountered at times in prompt reflooding of the fields, and, at least in the Mississippi Delta area, the vastly increased production of *Psorophora* which accompanies the periodic draining and reflooding.

Isley & Schwart (1932) considered the rice water weevil as the most destructive insect pest of rice in Arkansas but Douglas & Ingram (1942) state that in Louisiana this species is of no economic importance.

PROCEDURE.—All investigations were conducted in an area near Cleveland, Mississippi, where rice had been grown only since 1950. The chemical treatments designed chiefly for mosquito control included both pre- and post-flood applications. The former consisted of: (a) dieldrin emulsion: 0.5 and 1.0 lb. dieldrin/acre, 3 and 5 gal.

[1] From the Communicable Disease Center, Bureau of State Services, Public Health Service, U. S. Department of Health, Education, and Welfare, Savannah, Georgia. Accepted for publication July 15, 1958.

[2] Grateful acknowledgment is made to the Bolivar County Health Department and to the Mississippi State Board of Health for their cooperation in conducting these investigations.

finished spray/acre applied by airplane; or 0.25 and 0.75 lb. dieldrin/acre, 20 gal. finished spray/acre, ground applied by power sprayer. (b) dieldrin impregnated pellets: 1.0 lb. dieldrin/acre, pellets 5% dieldrin, applied by hand seeder. (c) DDT emulsion: 1 and 3 lb. DDT/acre, 20 gal. finished spray/acre, ground applied by power sprayer. All these treatments were applied to the seeded fields prior to their initial flooding.

The postflood applications were made by hand seeding of flooded plots with dieldrin-treated pregerminated seeds. The seeds were treated by submerging them in burlap bags in water for 24 hours and then removing and keeping the bags under a tarpaulin for an additional 24 hours. The moist seeds then were poured into a garbage can, and as this was being done, 25% dieldrin wettable powder was sifted over them. Four pounds of powder treated the amount of seeds required to plant 1 acre. Two days after planting, these plots were drained and left for approximately 1 week, then reflooded.

To evaluate the densities of adult weevils, the same device used for sampling larval mosquito populations was employed. It consisted of a metal cylinder (1 square foot) which was covered with hardware cloth ($\frac{1}{4}$" mesh) on one end (Horsfall 1942). To determine the degree of larval weevil infestation in a field, plants were selected at random from treated and untreated plots at weekly intervals. Each plant was removed by working a hand under its roots and then lifting so that roots and soil remained attached. Each group of five plants was placed in a separate container, all samples being carried to a central location for processing. In processing, all the soil was washed from the roots and strained through a set of three interlocking sieves (10–20, and 40-mesh) to recover the larvae. The washed roots also were examined for larvae and cocoons. Sampling in treated and untreated fields began 3 or 4 weeks after the initial flooding and continued until the population had completed its development. Approximately 50 plants per plot were sampled per inspection.

At harvest time, yields were obtained from plots treated by airplane with dieldrin emulsion at the rate of 0.5 and 1 pound of dieldrin per acre and from their respective untreated check plots.

RESULTS.—*Preflood Applications.*—In the 100-acre field treated on May 26 with dieldrin emulsion by airplane at a rate of 1 lb. of dieldrin per acre, adult weevils were numerous immediately after flooding occurred. Presumably, those adult weevils were migrants from adjacent areas. Twenty square-foot samples taken in 4 successive days from areas flooded the first week in both the treated and untreated fields showed a 69% reduction of the adult weevil population in the treated field.

Three weeks after the first flooding began, 50 plants were removed from the treated and untreated fields for the initial determination of larval and cocoon densities. Figure 1 gives the results of these and subsequent examinations.

In the untreated plot (A), the peak of infestation, based on the combined larval and cocoon densities, was reached during the sixth week after the initial flooding. By considering only the larvae recovered, this peak occurred during the fourth week; however, little difference was apparent among the larval infestations for the fourth, fifth, or sixth weeks. After this time, larval densities decreased rapidly. The first cocoon was detected during the fourth week with the peak of pupation being reached during the sixth week and gradually decreasing after this time. The 50 plants checked at the sixth week after flooding were washed individually. Larvae or cocoons were found on 48 plants, the highest number recovered from a single plant being 43. In contrast, only six larvae and one cocoon were recovered from the total of 400 plants sampled in the treated field.

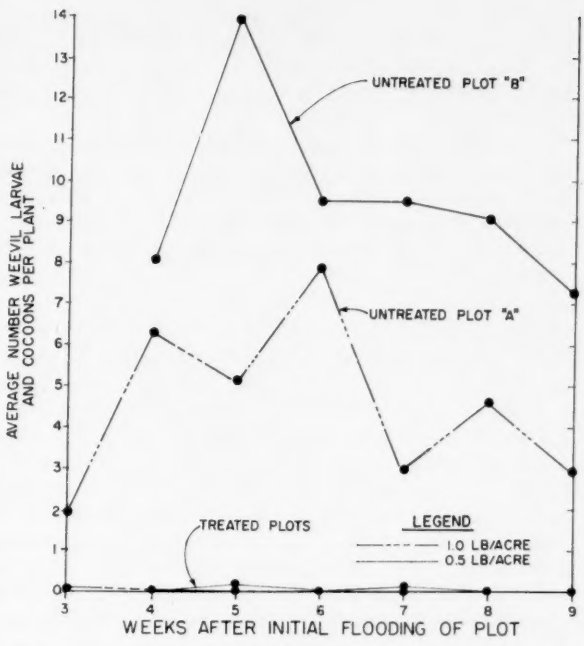

FIG. 1.—Comparison of densities of rice water weevil larvae and cocoons in fields receiving preflood applications of dieldrin emulsion at the rate of 0.5 pound or 1.0 pound of dieldrin per acre and in untreated fields (untreated plot A was check area for field treated with 1.0 pound of dieldrin per acre).

The results from the plot (48 acres) treated by airplane with 0.5 lb. dieldrin per acre were similar to those obtained with the 1.0 pound dieldrin/acre application (figure 1). The untreated plot (B, 70 acres) immediately adjacent to the treated field, showed a heavy larval infestation and a few cocoons on the first inspection 4 weeks after flooding; the peak infestation occurred the fifth week. The treated field yielded only two larvae and four cocoons on the 230 plants examined. Since some of the positive plants came from beneath a power line extending across the treated plot, a special sampling of 10 plants along this line was conducted. The data indicated an infestation rate of 6.5 larvae/cocoons per plant, this density presumable being a result of an insufficient treatment caused by the airplane rising to a height of 30 to 40 feet over the power line in contrast to the usual 6-foot elevation followed over the remainder of the field.

In the small plots (0.25 to 1.0 acre)treated with ground equipment, 10 plants per week were examined beginning 4 weeks after flooding. The area receiving 0.75 lb. dieldrin per acre showed only one larvae during the 7 weeks of inspection. From the same number of plants in the untreated plot, 145 larvae were recovered. Larvae or cocoons

were recovered each week on plants examined from the plot treated at the rate of 0.25 pound of dieldrin per acre, the densities being from 1 to 11 larvae/cocoons per plant as compared with 2 to 51 larvae/cocoons per plant in the untreated plot.

The single plot (4 acres) treated with dieldrin impregnated pellets yielded a few larvae in three of the seven inspections (10 to 20 plants/inspection) which occurred from the third to ninth week after it was flooded. A range of 0.3 to 0.8 larvae/cocoons per plant was recovered in the treated plot compared with 1.7 to 6.6 larvae/cocoons per plant in the untreated plot.

Neither of the DDT-treated rice plots (1 or 3 lbs. DDT/acre) showed any reduction in the degree of weevil infestation as compared with that of the untreated fields.

Postflood Application.—In each of the two plots (total 3.5 acres) seeded with dieldrin treated seeds, 60 plants were examined, the inspections being made from the fourth to ninth week after the flooding of the fields. The same number of plants were examined from an untreated plot that was planted in the conventional manner and flooded about the same time as the treated plots.

In the treated plots, a total of 4 to 6 larvae/cocoons per inspection were recovered whereas the check plot showed infestations which ranged from 4.4 larvae/cocoons per plant in the first examination to 18 larvae/cocoons per plant on the sixth and eighth weeks. The latter were the highest densities obtained from any untreated plots in these experiments.

In addition to evaluating the effect of the treatments upon weevil densities, comparisons were made of rice yields in certain treated and untreated plots. Comparative yields were measured only in fields planted to the same variety of rice on approximately the same date and on land that had been in rice for the same number of years. The field (100 acres) treated at the rate of 1 pound of dieldrin per acre with an emulsion formulation and its untreated plot (78 acres) were planted with Blue Bonnet Variety on land on which rice had been planted the previous year. The plot (48 acres) sprayed with dieldrin emulsion at 0.5 lb. of dieldrin per acre and its check plot (70 acres) was planted in Century Patna Variety on land that had never been in rice. Based on the weight of rice as it came from the field, the plot treated with 1.0 lb. of dieldrin/acre averaged 8 bushels more rice per acre than its untreated check plot (72 bu./acre). However, the average yield per acre from the plot treated with 0.5 pound of dieldrin per acre was approximately the same as that obtained from the untreated plot.

DISCUSSION.—The results of these tests indicate that preflood treatments of dieldrin emulsion (0.5 to 1.0 lb. of dieldrin/acre) essentially eliminated rice water weevil infestation from rice fields for periods of 9 and 10 weeks (length of entire observation interval). The same treatment also was highly effective against *Psorophora* larvae, but these mosquitoes developed resistance to the compound rapidly (Mathis *et al.* 1955). Nonetheless, the use of dieldrin for rice water weevil control indirectly could be a means of reducing *Psorophora* infestations by virtue of negating the need for the alternate flooding and drying practices now being employed for control of weevil damage. With permanently flooded rice fields, the production of successive broods of *Psorophora* would be remedied. The validity of such a conclusion, of course, is founded on the premise that the value of the irrigation cycle lies solely in its curtailment of weevil breeding.

In Texas, Bowling (1957) also reported dieldrin effective as a seed treatment against rice water weevil infestations. Similar results were obtained with lindane and aldrin.

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BOOK REVIEWS

DIE INSEKTIZIDE—CHEMIE, WIRKUNGSWEISE UND TOXIZITÄT, by Werner Perkow. viii+384 pp. incl. 16 illus. Dr. Alfred Hüthig Verlag, Heidelberg. 1956. Price clothbound, DM28.

Because the insecticide picture is such a dynamic one any text on the subject strikes one as being out of date by the time it reaches publication. The text is quite accurate as far as it goes, but one is left a bit disappointed perhaps because there is a tradition of extreme thoroughness in German texts which is not found in this one. It is also puzzling to this reviewer that Perkow has not included in this text any mention of his theories on correlating chemical structure with insecticidal activity. In sum this is a reasonably good text on insecticides for one who reads German. It contains a glossary of common German names of insects with their Latin equivalents.

S. A. HALL

(*Continued on page 36*)

Absorption and Metabolism of DDT in the Boll Weevil[1]

M. S. Blum,[2] N. W. Earle,[2] and J. S. Roussel[3]

Abstract

The basis for the natural tolerance of the boll weevil (*Anthonomus grandis* Boh.) for DDT was studied by treating weevils topically or by injection and determining the rate of penetration and metabolism of DDT. The weevil has a natural tolerance for DDT which varies with the strain, age, and time of year collected. Overwintered weevils are more sensitive than first-generation populations. Weevils resistant to chlorinated hydrocarbons, such as endrin, are slightly more resistant to DDT than endrin-susceptible weevils. Both resistant and susceptible weevils are very susceptible to injected dosages of DDT. The rates of absorption in susceptible and resistant boll weevils are equal; about 60% of a 5 μg. dosage being absorbed in 48 hours. Small amounts of DDT are found in both strains, but slightly more in the susceptible insects. DDE is produced in trace quantities by both strains. After 48 hours about 40% of the applied DDT is converted into unknown metabolites. Neither DDE nor DDA is involved in the primary avenue of DDT detoxication. Compounds reported as outstanding DDT synergists in the house fly increase the toxicity of DDT in the weevil only three- to five-fold. DDT is more toxic to the weevil at lower temperatures.

Certain insects never have been successfully controlled with DDT. This natural tolerance has been the subject of at least two laboratory investigations. Ferguson & Kearns (1949) demonstrated that the large milkweed bug (*Oncopeltus fasciatus* (Dall.)) could metabolize substantial quantities of DDT to unidentified products. They also showed that DDE, a suspected metabolite of DDT, was partially metabolized to an unidentified product, although the rate of DDE metabolism was slower than that of DDT. These authors concluded that metabolism to DDE was not a primary pathway of DDT degradation. They also showed that another suspected metabolite of DDT, DDA (2,2-bis(*p*-chlorophenyl) acetic acid), was rapidly metabolized, and therefore not an end product of DDT metabolism.

Sternburg & Kearns (1952) examined the penetration and metabolism of DDT in four naturally tolerant insects—the Mexican bean beetle (*Epilachna varivestis* Muls.), the red-banded leaf roller (*Argyrotaenia velutinana* (Wlk.)), the red-legged grasshopper (*Melanoplus femur-rubrum* (DeG.)), and the differential grasshopper (*M. differentialis* (Thos.)). They demonstrated that all these insects could metabolize DDT as well as DDE to unidentified compounds. Their tolerance for DDT was associated with this metabolism.

The boll weevil (*Anthonomus grandis* Boh.) was first reported by McGovran (1944) to be controlled inadequately with DDT. The development of the weevil's resistance to other chlorinated hydrocarbons (Roussel & Clower 1957) has emphasized the need for a better understanding of the toxicology and physiology of this insect. To gain further information, a study was made of the toxicity of DDT to different strains of weevils and of the susceptibilities of weevils of different ages and generations. The level of resistance to endrin was similarly examined in order to determine whether a cross-tolerance to DDT was present. When it had been demonstrated that susceptibility varied with age, the absorption rate of DDT was determined in weevils of different ages. The metabolism of DDT and two of its suspected breakdown products was examined in resistant and susceptible weevils in order to determine the importance of metabolism to DDT resistance. In addition, certain DDT analogues, reported to be excellent synergists for DDT in the control of the house fly, were tested as DDT synergists against the weevil. The relationship between temperature and DDT toxicity also was examined.

General Procedure.—Overwintered weevils were collected from seedling cotton in the spring; all others were reared from infested squares picked from cotton in the field. Adult weevils were fed blooms until the time of treatment.

For dosage-mortality studies, insecticides were applied by treating weighed boll weevils on the dorsal surface of the metathorax with 1 microliter of an acetone solution. The insecticide was administered with a calibrated 0.25-ml. syringe, the plunger of which was driven by a micrometer. After treatment, groups of five weevils were placed in half-pint jelly jars with perforated lids and containing cotton squares. Mortality counts were made after 24 and 48 hours. Dosage-mortality curves were plotted on log-probability paper, and LD-50 values were computed by slope determination of the response curves.

The same method of treatment was used for metabolism studies, but the treated insects were held in 125-ml. Erlenmeyer flasks without food.

DDT or its analogues were prepared for injection by mixing 1 ml. of an acetone solution with 9 ml. of a 1% aqueous solution of Tween 80 (a mixture of polyoxyethylene sorbitan mono-oleates). A small hole was punched into the thorax with a fine pin, and 1 microliter of suspension was injected with a sharpened No. 30 needle, delivery of the solution being made in the usual manner. After treatment, the hole was sealed with melted embedding paraffin applied by means of a heated hypodermic needle.

The following chemicals were used in this study:

p,p' DDT—2,2-bis(*p*-chlorophenyl)-1,1,1-trichloroethane, recrystallized twice from ethanol; m.p. 108°–109° C.

p,p' DDE—2,2-bis(*p*-chlorophenyl)-1,1-dichloroethylene, obtained by dehydrochlorination of DDT in alcoholic potassium hydroxide and recrystallized twice from ethanol; m.p. 88°–89° C.

*p,p'*DDA—2,2-bis(*p*-chlorophenyl)acetic acid, prepared

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[2] Entomology Research Division, Agricultural Research Service, U. S. Department of Agriculture.

[3] Louisiana Agricultural Experiment Station.

Table 1.—LD-50 values for DDT and endrin. Weevils of different ages and from different areas in Louisiana.

Source of Weevils	Generation and Age (Days)	LD-50 (μg./Gm.) DDT	Endrin
Topical Application (48-hour mortality)			
Chambers	Overwintered	860	7
	First, 4	2000	26
Lafayette	Overwintered	—	1.1
	First, 3	—	1.5
Eagle Lake	First, 1	980	—
	2	1700	—
	3	2600	—
	4	3200	—
	5[a]	2700	26
Injection (24-hour mortality)			
Chambers	Overwintered	52	—
Lafayette	First, 3	33	—

[a] For DDT; second generation, 4 days for endrin.

by alcoholysis of DDT in a potassium hydroxide solution in ethylene glycol; m.p. 165°–167° C.

1,1-bis(*p*-chlorophenyl)ethane, prepared by the condensation of paraldehyde with chlorobenzene (Dominion Tar and Chemical Co., Ltd., 1952).

1,1-bis(*p*-chlorophenyl)-2,2,2-trifluoroethanol.[4]

Endrin, technical—1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-endoendo-5,6-dimethanonaphthalene.

Toxicity of DDT to Different Strains of Weevils.—The LD-50 values for DDT and endrin for boll weevils from three locations in Louisiana are given in table 1. Weevils from three generations are included. The topical mortality data are 48-hour determinations. This period was selected because the mortalities resulting from treatment with DDT and endrin approached their maximum values in this time. Mortalities resulting from the injection of DDT reached their maxima in less than 24 hours.

The weevils from Chambers and Eagle Lake were found to be fairly resistant to endrin. The Lafayette weevils were predominately susceptible. Both DDT and endrin were more than twice as toxic to the overwintered than to the first-generation Chambers weevils. Endrin was slightly more toxic to the overwintered than to the first-generation Lafayette weevils. The Lafayette strain was the most susceptible to both endrin and DDT. These data demonstrate that resistance to endrin and related chlorinated hydrocarbons is associated with resistance to DDT. This correlation parallels the demonstration by Decker & Bruce (1952) that house flies selected for resistance to dieldrin were slightly more resistant to DDT.

Relation Between Age, Susceptibility to DDT, and Rate of Absorption of DDT.—The weevil's resistance to DDT increased with its age. In the Eagle Lake strain the greatest difference appeared between 1- and 2-day-old weevils (table 1). Since the cuticle of such weevils is softer than that of older weevils, it seemed possible that DDT might penetrate it more easily. Penetration studies were performed on groups of 15 weevils. Two series of four replicates each were usually employed for each time interval. After the treated groups had been held for different time intervals at 28° C., the weevils were given three successive 15-ml. rinses with distilled ethyl ether. The ether was evaporated on a steam bath, and the samples were analyzed by the method of Schechter *et al.* (1945) to obtain the amounts of DDT remaining on the external surfaces of the weevils ("external rinse"). The data for several time increments in weevils 1 to 5 days old are summarized in table 2. Initially slightly more DDT was absorbed by the 1- and 2-day-old weevils than by the older weevils. However, after 24 hours the differences were found to be considerably less. It seems unlikely, therefore, that the greater susceptibilities of young weevils could be due to a more rapid penetration of toxicant.

Table 2.—Per cent of applied dosage (5 μg.) of DDT recovered from external surface of boll weevils of different ages at different intervals after treatment.

Age of Weevils (Days)	Interval After Treatment Immediate	2 Hours	7 Hours	24 Hours
1	98	77	56	40
2	99	78	57	40
3	99	80	61	41
4	99	82	63	43
5	99	82	63	42

The cuticle does appear to serve as a protective barrier; however, DDT was found to be highly toxic to the weevil when injected (table 1). This would indicate that the relative inactivity of DDT by topical application is due to its failure to reach the site of action. The sensitivity of the weevils was further reflected in the speed of action of injected DDT. Immediately after injection they became excited and walked backwards. Paralysis followed rapidly.

Relation Between Weevil Resistance and Ability to Detoxify DDT.—The penetration rates of topically applied DDT in a resistant (Eagle Lake) and a susceptible (Lafayette) strain were compared. The results show that there is little difference between the two strains (table 3). Resistance is therefore not due to the inability of DDT to penetrate the cuticle.

The possibility that detoxification was causally related with resistance was then considered. The fate of topically applied DDT was studied in weevils held at 28° C. At various intervals after treatment, groups of weevils were rinsed with ether to recover the unabsorbed DDT. The rinsed weevils were then ground in a mortar with anhydrous sodium sulfate. The resulting powder was transferred to a 4-ounce, screw-cap bottle with the aid of a small portion of ether and the mixture mechanically agitated with three 15-ml. portions of ether for 1 hour. The three portions were filtered and combined to give the "internal extract." After evaporation of the ether, DDT and two possible breakdown products, DDE and DDA, were analyzed by the method of Schechter *et al.* (1945). Mixtures of DDT and DDE were analyzed by the procedure of Mattson *et al.* (1953).

[4] Obtained through the courtesy of E. B. Bergmann, Hebrew University Jerusalem, Israel.

The amounts of DDT and DDE recovered from the internal extracts of DDT-treated resistant and susceptible weevils are presented in table 3. The steady disappearance of DDT parallels its rate of absorption throughout the 48-hour period. No DDA and only small amounts of DDE were recovered from weevils of either strain. DDT apparently was converted into compounds not responding to the Schechter-Haller colorimetric method. After 24 and 48 hours more DDT was found in the internal extracts of susceptible weevils than from extracts of resistant weevils. However, the difference was only slight and could not account for the higher mortality of the susceptible weevils.

Table 3.—Recovery of DDT and DDE in the external rinses and internal extracts of 4-day-old resistant and susceptible boll weevils treated topically with 5 μg. of DDT.

Hours After Treatment	Per Cent of Applied Dosage Recovered			Per Cent of Absorbed DDT Metabolized
	External Rinse	Internal Extract		
	DDT	DDT	DDE	
Resistant (4 to 8% mortality after 48 hours)				
0	99	—	—	—
2	81	6.8	0	64
7	62	13	Trace	66
24	53	6.2	2.4	77
48	43	11	3.4	80
Susceptible (24 to 28% mortality after 48 hours)				
0	98	—	—	—
2	82	8.4	0	53
7	64	12	0	66
24	56	14	1.2	67
48	44	16	2.2	72

Neither DDT nor DDE appeared to be excreted by the weevils. In only two cases were traces of DDT found in the excreta, and it seems likely that this represented a portion of the unabsorbed DDT that had been accidentally removed from the outside of the insects.

PATHWAY OF DDT DETOXIFICATION.—The appearance of small amounts of DDE in the internal extracts of DDT-treated weevils suggested that it might be an intermediate in the metabolism of DDT. This possibility was examined by studying the absorption and metabolism of DDE in resistant weevils. As shown in table 4, DDE penetrated at a rate nearly equal to that of DDT (table 3). Nearly 70% of the absorbed DDE was recovered, demonstrating that it could not be an important intermediate in the metabolism of DDT. Recovery of topically applied DDA was near theoretical, eliminating this compound also as a possible intermediate in the metabolism of DDT.

Of the naturally resistant insects which have been studied, the boll weevil most closely resembles the milkweed bug examined by Ferguson & Kearns (1949). Both insects convert DDT to a product(s) not responding to the Schechter-Haller method, and both insects have a capacity for DDE metabolism. In neither case is DDE an intermediate in the metabolism of DDT. However, whereas DDA is rapidly metabolized by the milkweed bug, the boll weevil does not degrade this compound. The boll weevil also resembles the two species of grasshoppers examined by Sternburg & Kearns (1952) in being tolerant to large dosages of topically applied DDT and susceptible to relatively small injected dosages. However, whereas the grasshoppers never contained DDT inside the body, the boll weevil has been shown to contain significant amounts.

Table 4.—Recovery of DDE and DDA in the external rinses and internal extracts of 4-day-old resistant boll weevils topically treated with 5 μg. of each compound.

Hours After Treatment	Per Cent of Applied Dosage Recovered		Per Cent of Absorbed Material Metabolized
	External Rinse	Internal Extract	
DDE (2 to 4% mortality after 48 hours)			
0	99	—	—
2	82	12	35
7	64	27	24
24	56	34	24
48	38	43	31
DDA (3 to 5% mortality after 48 hours)			
0	98	—	—
24	81	16	6.4

TOXICITY OF DDT-SYNERGIST COMBINATIONS.—DDT-resistant house flies are susceptible to combinations of DDT and certain structural analogues (Summerford *et al.* 1951). Furthermore, Dieter & Thiegs (1950) have demonstrated that DDT can be synergized in a naturally tolerant insect, the Mexican bean beetle, by one of its structural analogues. Consequently, the capacity of certain DDT analogues to synergize DDT in the boll weevil was determined, using overwintered susceptible, and first generation resistant weevils. Two of the most effective house fly synergists for DDT were selected: 1,1-bis(*p*-chlorophenyl)ethane (March *et al.* 1953) and 1,1-bis(*p*-chlorophenyl)-2,2,2-trifluoroethanol (Tahori 1955). Each synergist was applied topically with DDT in a 1:1 ratio at three dosage levels. In the house fly maximum synergistic activity was demonstrated with this ratio of DDT tos ynergist. Results showed that the toxicity of DDT to either weevil strain was increased only by a factor of three to five when the synergist was present. The ethane analogue itself was about as toxic as DDT to the weevils, whereas the fluoroethanol analogue was essentially nontoxic.

The possibility that the synergist did not penetrate rapidly enough to exert its effect was examined by injecting DDT-synergist combinations. Again the toxicity of DDT was increased by a factor of only about threefold.

It is interesting to note that DDT can be synergized in the Mexican bean beetle, which can dehydrochlorinate DDT to produce DDE. It is possible that DDT synergists are most effective in those insects utilizing dehydrochlorination as the primary avenue of DDT detoxification. This view is strengthened by the demonstration by Moorefield & Kearns (1955) that DDT synergists are competitive inhibitors for the enzyme that dehydrochlorinates DDT in the house fly. Since the boll weevil metabolizes only small amounts of DDT to DDE, DDT synergists would not be blocking the main pathway of DDT detoxification.

RELATION BETWEEN TEMPERATURE AND DDT TOXICITY.—Hoffman & Lindquist (1949) observed that DDT was most toxic to house flies at low temperatures. The response of DDT-treated weevils has been found to be the same. Weevils treated with 5 micrograms of DDT at 28° C. showed symptoms of poisoning and became immobilized as soon as they were transferred to 5° or 15° C. either 12, or 24, or 48 hours after treatment. Paralysis occurred at 5° C. within minutes after being transferred to this temperature. A return to near normalcy occurred shortly after the weevils were returned to 28° C., but the tremors symptomatic of DDT poisoning persisted for some time.

The negative temperature coefficient of DDT in the boll weevil is probably due to the supression of the toxicity of absorbed DDT by high temperatures. This parallels the phenomenon found in the American cockroach by Vinson & Kearns (1952).

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Biological Activity and Metabolism of Hercules AC-528 Components in Rats and Cockroaches[1,2]

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ABSTRACT

Technical Hercules AC-528 (Delnav) was separated by partition chromatography into eight different fractions. The major components were the *cis* and *trans* isomers of 2,3-*p*-dioxanedithiol *S,S*-bis(*O,O*-diethyl phosphorodithioate). The structure, toxicity to house flies and rats, anticholinesterase activity and stability to alkaline hydrolysis were studied for these eight Hercules AC-528 components. Radioactive Hercules AC-528 was prepared and the metabolism in rats and cockroaches studied for the *cis* and *trans* isomers, 2-*p*-dioxenethiol *S*-(*O,O*-diethyl phosphorodithioate) and bis(diethoxyphosphinothioyl) disulfide. In a wide variety of *in vivo* and *in vitro* biological systems the *cis* and *trans* isomers were the most stable of the radioactive components; the single exception was a study on hydrolysis by human plasma where the dioxene derivative was the most stable. In a sub-acute feeding study with rats, Hercules AC-528 was found to accumulate to a small degree in fat. However, the residues disappeared rapidly when feeding of Hercules AC-528 was discontinued.

Other factors in investigating Hercules AC-528 included: cholinesterase depression and recovery in rats following administration of a sub-lethal dose; the effect of sub-acute feeding on rat plasma, red blood corpuscle and brain cholinesterase activity; metabolism of the components of Hercules AC-528 by *Periplaneta americana* (L.) and rat liver slices; the formation of more polar, non-hydrolyzed metabolites from the radioactive components by rats and cockroaches; and the nature of the hydrolysis products formed from the components in human plasma and following oral administration to rats.

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[2] A portion of a dissertation submitted by the senior author to the Graduate Faculty of the University of Wisconsin in partial fulfillment of the requirements for the Ph.D. degree.

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Hercules AC-528 (Delnav) is an acaricide and insecticide of long residual effectiveness (Diveley & Lohr 1955). It is a mixture of related phosphorothioates with 70% of the technical product consisting of the *cis* and *trans* isomers of 2,3-*p*-dioxanedithiol *S,S*-bis(*O,O*-diethyl phosphorodithioate) (Diveley *et al.* 1958). A colorimetric method is available for determining these isomers in microgram quantities (Dunn 1958) .These investigations deal with the biological activity and metabolism of Hercules AC-528 components in rats and cockroaches.

CHEMICAL STUDIES.—*Composition of Technical Hercules* AC-528.—Elemental analysis of technical Hercules AC-528 gave a close approximation to theory for 2,3-*p*-dioxanedithiol *S,S*-bis(*O,O*-diethyl phosphorodithioate) (table 1).

Eight different fractions were obtained by partition chromatography of technical Hercules AC-528 (fig. 1). The chromatographic procedure was similar to that described by Diveley *et al.* (1958) using a celite base and acetonitrile equilibrated with *n*-heptane as the stationary phase and *n*-heptane equilibrated with acetonitrile as the mobile phase. The solvent pair, *n*-heptane-acetonitrile, gave incomplete resolution of these fractions but was superior to acetonitrile with *n*-hexane, Skelly-B or Skelly-C, or methanol with *iso*octane or Skelly-C. Fractions I (a, b and c), II, III and IV were eluted with *n*-heptane equilibrated with acetonitrile, fraction V with acetonitrile equilibrated with *n*-heptane and fraction VI with methanol alone. The per cent of the various components in technical Hercules AC-528 based both on weight and total phosphorus (Allen 1940) is shown in table 1.

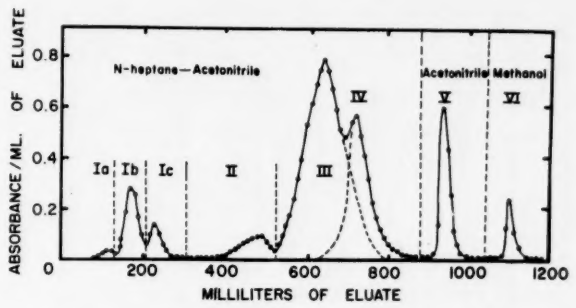

Fig. 1.—Chromatogram of 250 mg. technical Hercules AC-528 on 35 gm. celite. (The mobile phase solvents indicated are *n*-heptane equilibrated with acetonitrile, then acetonitrile equilibrated with *n*-heptane and finally methanol only.)

Fraction I appears to consist of a mixture of ethyl phosphorothioates. Their structure is considered in more detail in a separate section. Elemental analysis of fraction II agrees well with theory for 2-*p*-dioxenethiol *S*-(*O,O*-diethyl phosphorodithioate) and the infrared spectrum (fig. 2) is identical to that for the known dioxene deriva-

Table 1.—Composition of technical Hercules AC-528 and properties of components.

	Technical Hercules AC-528	Fractions Eluted from Celite Column[a]							
		Ia	Ib	Ic	II	III	IV	V	VI
Per cent of technical Hercules AC-528									
weight	100	0.3	6.8	3.0	6.4	—68.3—		12.0	3.2
total phosphorus	100	0.5	7.2	2.7	5.6	49.7	23.5	8.2	2.6
Elemental analyses (*per cent*)									
Phosphorus									
calculated	13.6	—	—	—	11.5	13.6	13.6	—	—
found	13.4	12.5	15.8	12.6	10.4	11.7	12.7	7.3	7.8
Sulfur									
calculated	28.1	—	—	—	23.7	28.1	28.1	—	—
found	27.6	23.0	31.9	27.0	23.6	25.6	28.3	22.7	25.1
Carbon									
calculated	31.6	—	—	—	35.6	31.6	31.6	—	—
found	32.5	—	30.6	33.3	35.6	33.1	32.5	29.2	32.6
Hydrogen									
calculated	5.7	—	—	—	5.6	5.7	5.7	—	—
found	5.7	—	5.9	6.3	5.7	6.1	6.2	5.1	5.2
Chlorine found	0.9	—	—	4.9	1.2	1.9	0.7	3.0	2.8
Biological activity									
LD_{50} rats, mg./kg.[b]	95	135	75	80	90	240	65	—450	—
LD_{50} house flies, mg./kg.[c]	15	40	15	34	12	21	7	110	170
P.p.m. for 50% ChE inhibition[d]	10	8	6	6	112	100	>320	—5—	
Alkaline stability-$T_{1/2(min.)}$[e]	150	<0.5	33	50	1.2	188	182	—<0.5—	

[a] Column fractions as indicated in figure 1, with infrared spectra shown in figure 2. Fraction II is the dioxene derivative, fraction III the *trans* isomer of Hercules AC-528 and fraction IV the *cis* isomer of Hercules AC-528.

[b] Subcutaneous administration in corn oil solutions to 125- to 150-gram male white rats with mortality recorded after 48 hours.

[c] Topical application in acetone to female house flies and mortality recorded after 24 hours (method of Krueger & Casida 1957).

[d] The p.p.m. of each component required to inhibit the cholinesterase activity of 30% whole human blood by 50% after pre-incubation at 37° C. for 1 hour prior to addition of substrate (method of Casida *et al.* 1956).

[e] Half-life in 10% ethano. plus 90% 1.0 molar sodium hydroxide.

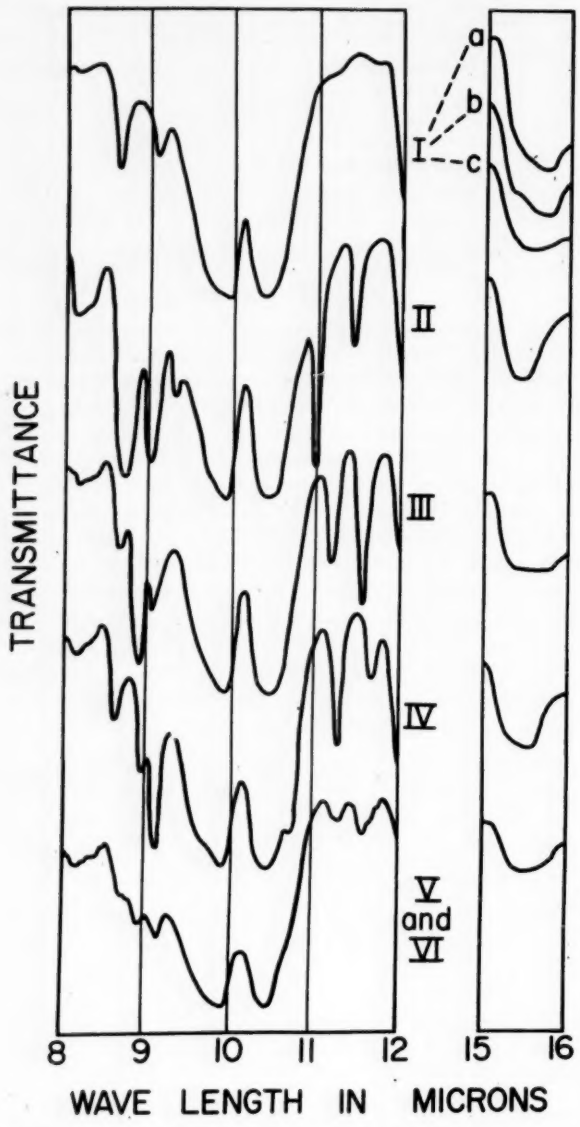

Fig. 2.—Partial infrared spectra on Hercules AC-528 components (Baird spectrophotometer, sodium chloride prisms, with compounds as 10% solutions in chloroform).

tive (Diveley *et al.* 1958). Elemental analyses on fractions III and IV were similar and approximated theoretical for 2,3-*p*-dioxanedithiol *S,S*-bis(*O,O*-diethyl phosphorodithioate). The isomeric assignment of Diveley *et al.* (1958) was followed with the fraction III being designated as *trans* and the fraction IV as *cis*. Fractions V and VI appeared to be mixtures of compounds with high chlorine content in certain constituents. Fraction V, although eluting in a homogenous manner from the celite column, was resolved into four separate phosphorus-containing fractions by paper chromatography (system of Cook 1954). Fractions V and VI were generally considered together since both could be eluted from the celite column with methanol, if acetonitrile was not used as a preliminary solvent after the *n*-heptane equilibrated with acetonitrile. Identification of the components of fractions V and VI was not attempted.

The major Hercules AC-528 components can be easily differentiated by their infrared spectra. The best peaks for characterization are in the regions of 8.5 to 9.5 microns and 10.9 to 11.9 microns as shown in figure 2. All the fractions from technical Hercules AC-528 had absorption peaks from 3.3 to 3.5 microns and at 6.80, 6.95 and 7.20 microns. The three components of fraction I (a, b and c) were almost identical in their spectra with only slight differences in the region of 15 to 16 microns. In addition to the absorption peaks shown in figure 2, fraction II had peaks at 6.15, 6.90, 7.30, 7.68 and 7.90 microns; fraction III and IV had peaks at 7.55 to 7.60 microns and at 7.90 microns; and the combined fractions V and VI had a 5.80 absorption peak.

Stability to Alkaline Hydrolysis and Thermal Degradation.—The stability of technical Hercules AC-528 and its components was determined in strong alkali. The organophosphates were dissolved in 95% ethanol at 5.0 mg. per ml. and the ethanol solution was added to nine times its volume of 1.0 molar aqueous sodium hydroxide. At frequent intervals 3.0-ml. aliquots were withdrawn, partitioned with 3.0 ml. chloroform and the total phosphorus content (Allen 1940) of each phase determined. With the pure Hercules AC-528 components a straight line resulted from plotting the logarithm of the per cent of the total phosphorus recovered in the chloroform layer against time.

Technical Hercules AC-528 was found to have 17% of its total phosphorus as materials which were hydrolyzed by alkali with a half-life of less than 2 minutes, based on the change in slope of the hydrolysis curve. These less stable components were due to fractions Ia, II, and V plus VI and their total amount agrees quite well with their proportionate composition in the technical material and their stability (table 1). The most stable components were the *cis* (IV) and *trans* (III) isomers which displayed identical half-lives of 182 to 188 minutes under these conditions. When fraction V was studied separately it was found to have a half-life of less than 0.5 minutes, while the combination of V plus VI contained 20 to 30% of more stable components which had an overall half-life of 500 to 600 minutes. The less stable components of V and VI are therefore eluted primarily in fraction V. Fraction Ia consisted of 96% of a material with a half-life of less than 0.5 minutes with the remaining 4% having a half-life of 100 to 200 minutes. Fractions Ib and Ic were almost identical based on hydrolysis curves with about 38% of an unstable material with a half-life of less than 0.5 minutes and a half-life of about 200 minutes for the most stable components.

The dioxene derivative (fraction II) is believed to arise from dehydrochlorination of *cis*-3-chloro-2-*p*-dioxanethiol *S*-(*O,O*-diethyl phosphorodithioate) (Diveley *et al.* 1958). Incubation of the *trans* isomer of Hercules AC-528 at 1000 p.p.m. in 0.1 molar sodium bicarbonate until 15% hydrolysis occurred yielded none of the dioxene derivative based on chromatographic separation of products. Near quantitative formation of the dioxene derivative from *trans*-Hercules AC-528 was obtained by pyrolysis at 145° C. for 45 minutes. An infrared spectrum of a purified sample of this pyrolysis product was identical to

that for the known dioxene derivative. The *cis* isomer of Hercules AC-528 was not decomposed by a similar pyrolysis. These pyrolysis results confirm the observations of Diveley *et al.* (1958).

Constituents of Hercules AC-528 Fraction I.—Fraction I of technical Hercules AC-528 consists of at least three components which cannot be completely resolved on the celite-*n*-heptane-acetonitrile column (fig. 1). These three materials were almost identical in infrared spectra with only slight changes in the region of 15 to 16 microns (fig. 2). Elemental analyses of the three fractions (table 1) yielded a sulfur to phosphorus ratio of 1.8 for Ia, 2.0 for Ib and 2.1 for Ic. All three fractions were eluted from the celite column with *n*-heptane equilibrated with acetonitrile. Elution from an alumina column (Woelm, chromatographic grade aluminum oxide with 2.0% water added according to Dunn 1958) occurred primarily in the benzene fraction with trace amounts in the *n*-hexane fraction. These observations, combined with the lack of an infrared absorption peak in the P=O region of 8.0 to 8.1 microns, indicate that all three components are phosphorothioates and probably phosphorodithioates.

Five phosphorothioates considered as possible components of fraction I from technical Hercules AC-528 were compared with fractions Ia, Ib and Ic. *O,O,O*-triethyl phosphorothioate and *O,O,S*-triethyl phosphorodithioate were proposed by Dively *et al.* (1958) as possible components of fraction I. In addition to these materials, sulfotepp, bis(diethoxyphosphinothioyl) disulfide, and the anhydride, $(C_2H_5O)_2P(S)SP(S)(OC_2H_5)_2$, of diethyl phosphorodithioic acid were also studied. All five of these ethyl phosphorothioates were found to elute from the celite column with *n*-heptane equilibrated with acetonitrile in a similar position to the fraction I components, and from the alumina column with benzene with trace amounts in the *n*-hexane fraction. Infrared spectra on these five compounds were all quite similar to the fraction I components. A 10.7 micron absorption peak in sulfotepp was absent in the fraction I components. The absorption band between 15 and 16 microns with Ia, Ib and Ic was present at 15.7 microns in bis(diethoxyphosphinothioyl) disulfide and at 15.4 microns in *O,O,S*-triethyl phosphorodithioate but was absent in the other three materials. On the celite column the disulfide was eluted first, then a mixture of the anhydride and *O,O,O*-triethyl phosphorothioate, and finally a mixture of sulfotepp and *O,O,S*-triethyl phosphorodithioate. The LD_{50} to house flies in μg./gm. and hydrolysis half-life in 0.1 N. sodium hydroxide under the previously described conditions were as follows for the five ethyl phosphorothioates: *O,O,O*-triethyl phosphorothioate—2000 μg. and over 4800 minutes; *O,O,S*-triethyl phosphorodithioate—44 μg. and 34 minutes; sulfotepp—1.3 μg. and 8 minutes; bis(diethoxyphosphinothioyl) disulfide—greater than 4000 μg. and less than 0.5 minutes; and the anhydride, $(C_2H_5O)_2P(S)SP(S)(OC_2H_5)_2$—20 μg. and 5 minutes.

The overall results indicate that *O,O,O*-triethyl phosphorothioate and sulfotepp are not constituents of fraction I. Fraction Ia appears to be bis(diethoxyphosphinothioyl) disulfide with about 4% of a more stable component. Fractions Ib and Ic are mixtures with the less stable component similar in stability to the disulfide and the other components more stable than either *O,O,S*-triethyl phosphorodithioate or the anhydride $(C_2H_5O)_2P(S)SP)(OC_2H_5)_2$. It is possible that fraction Ib might contain some anhydride and Ic some *O,O,S*-triethyl phosphorodithioate. The data do not allow an assignment of structure to the components of fractions Ib and Ic other than that they are ethyl phosphorothioates and probably ethyl phosphorodithioates.

Synthesis of Hercules AC-528-P^{32} and Characterization of Components: Hercules AC-528-P^{32} was prepared from phosphorus-^{32} pentasulfide (4 weeks service irradiation at Oak Ridge, Tennessee at 7×10^{11} neutrons per sq. cm. per second). The phosphorus pentasulfide was suspended in benzene and four molar equivalents of anhydrous ethanol added to form *O,O*-diethyl hydrogen phosphorodithioate. Two molar equivalents of this phosphorodithioate were then reacted with 2,3-dichloro-*p*-dioxane in benzene in the presence of a zinc chloride catalyst (Diveley *et al.* 1958) to yield Hercules AC-528-P^{32} with a specific activity of about 5 millicuries per gram. In several laboratory syntheses the proportions of *cis*, *trans* and dioxene derivatives varied considerably. Partition chromatography of the technical Hercules AC-528-P^{32} which most closely approximated the technical in infrared spectrum yielded 74.8% of *cis* and *trans* isomers, 5.3% dioxene derivative, 8.5% fraction I and 11.4% fractions V plus VI. Each fraction was re-chromatographed and that portion having an infrared spectrum identical to the known pure components was used in the metabolism studies. The fraction I-P^{32} was identical to bis(diethoxyphosphinothioyl) disulfide in elution position from the celite and alumina columns, infrared spectra with an absorption band at 15.7 microns, and in the very rapid hydrolysis by aqueous alkali.

TOXICITY OF TECHNICAL HERCULES AC-528 AND ITS COMPONENTS.—*Toxicity to Rats, House Flies and Anticholinesterase Activity of Hercules AC-528 Components.*—The most toxic component was the *cis* isomer (fraction IV) (table 1). *Cis*-Hercules AC-528 was three to four times more toxic than *trans*-Hercules AC-528 (fraction III) to house flies (*Musca domestica* L.) and white rats. Fractions Ia, Ib, Ic and II were also quite toxic but were present in lower percentages in the technical material. The anticholinesterase activity was least with the *cis*, *trans* and dioxene derivatives and greatest with fractions Ib, Ic and V plus VI.

Cholinesterase Depression and Recovery in Rats Following Technical Hercules AC-528 Administration.—Technical Hercules AC-528 in corn oil was administered subcutaneously at 75 mg. per kg. to male white rats weighing 125 to 150 gm. each. Rats were sacrificed and the brain, plasma and red blood corpuscle cholinesterase activity determined at 0, 0.5, 2, 4, 8 and 16 hours and 1, 2, 3, 5 and 7 days after treatment. Determinations were made manometrically (DuBois *et al.* 1950) with acetylcholine chloride at a final concentration of 0.05 molar. Brain homogenates were assayed at 2.5% and the plasma and corpuscles each at 10% final concentration. The rats displayed muscular fibrillation at 2 hours, convulsions at 4 to 8 hours, and the symptoms of organophosphate poisoning continued several days before recovery. The plasma cholinesterase activity was depressed 60% and the corpuscle activity by 28% within 30 minutes. At 2 hours the brain cholinesterase activity was still normal as was

Table 2.—Effect of oral administration of technical Hercules AC-528 on cholinesterase activity in brain, red blood corpuscles and plasma and the residues of Hercules AC-528 in the fat of rats.

Treatment	Interval in Days[a]	Per Cent ACh Esterase Inhibition						P.p.m. in Fat[b]	
		Brain		Corpuscle		Plasma			
		♂	♀	♂	♀	♂	♀	♂	♀
5 mg./kg. technical	1	4	14	23	30	18	21	0.0	0.0
	3	10	34	53	68	55	65	0.2	0.6
	7	31	48	75	77	49	66	0.5	0.6
	21	46	70	81	73	43	64	0.2	0.6
	7 on+10 off	14	31	4[illegible]	43	6	+40	0.1	0.0
10 mg./kg. technical	7	62	75	86	82	62	83	0.4	0.4
2.5 mg./kg. *Cis*	7	17	33	64	63	17	41	−0.2	−0.1

[a] Oral doses administered in corn oil daily for the interval indicated and the rats sacrificed 24 hours after the last dose except in one case rats sacrificed 10 day after the last dose.
[b] Average of duplicate analyses made by Dr. C. L. Dunn, Hercules Powder Company.

the activity of brain homogenates on triacetin (0.02 molar) and *o*-nitrophenyl acetate (0.03 molar). Maximum cholinesterase inhibition of 85, 78 and 73% occurred at 16 hours with the plasma, corpuscle and brain, respectively. Very little recovery in cholinesterase activity occurred by 3 days, and even at 7 days the activity of the enzymes was 40 to 60% inhibited. The corpuscle cholinesterase was the slowest to recover.

Oral administration of technical Hercules AC-528 at 5.0 mg. per kg. per day resulted in plasma, red blood corpuscle and brain cholinesterase inhibition within 1 day (table 2). Between the seventh and 21st day of subacute feeding, little further inhibition resulted with the plasma and red blood corpuscle cholinesterase but the inhibition of brain cholinesterase greatly increased. Recovery of cholinesterase activity was most rapid with the plasma. The female rats were considerably more sensitive than the male rats. These observations with technical Hercules AC-528 are in general agreement with the results of Frawley *et al.* (1952) on toxicity and cholinesterase inhibition in rats poisoned with parathion, EPN and Potasan.

Cis-Hercules AC-528, the component of technical Hercules AC-528 with the highest acute toxicity, was not the only component yielding toxic effects on sub-acute feeding. When the *cis* isomer was fed to rats at the same level as its proportionate composition in the technical material (*i.e.* 2.5 mg. per kg. *cis* versus 10 mg. per kg. technical), the cholinesterase depression was much greater with the technical Hercules AC-528. The acute and subacute mammalian toxicity of technical Hercules AC-528 is therefore due to more than one of its components.

Metabolism and Residue Studies with Rats and Cockroaches.—*Studies with Hercules AC-528-P*^{32} *in Rats.*—Female white rats (two replicates of two 160- to 180-gram rats each) were treated orally with technical Hercules AC-528-P^{32} and certain of its separate components. The rats were held in metabolism cages which allowed for the separation of urine and feces (Comar 1955). Rats treated orally for 10 consecutive days at 1 and 5 μg. technical Hercules AC-528-P^{32} per kg. daily excreted Hercules AC-528 metabolites primarily in the urine and to a lesser extent in the feces (fig. 3). The relative excretion rates of the two dosage levels were very similar. After the first 5 days there was an equilibrium between the radioactivity administered and the radioactivity excreted each day. More than 98% of the radioactivity in the urine was in the form of hydrolysis products.

Rats treated orally at 25 mg./kg. with certain components of radioactive Hercules AC-528 were sacrificed 48 hours after treatment. The total p.p.m. of radioactive materials and the total p.p.m. of *n*-hexane-soluble radioactivity occurring in several tissues were determined (table 3). The tissues were thoroughly macerated in a mortar and pestle with white sand and *n*-hexane. The *n*-hexane extracts were then dried with anhydrous sodium sulfate. The kidney and liver contained a higher concentration of radioactive materials than other tissues but most of this radioactivity was not extractable into *n*-hexane. The fat from rats treated with the *cis* and *trans* isomers was the only tissue having appreciable amounts of *n*-hexane-soluble radioactive materials. This *n*-hexane-soluble radioactivity from the fat was recovered as the administered chemical based on partition chromatography with known carrier compounds (table 3).

The proportion of a 25-mg./kg. dose excreted in the

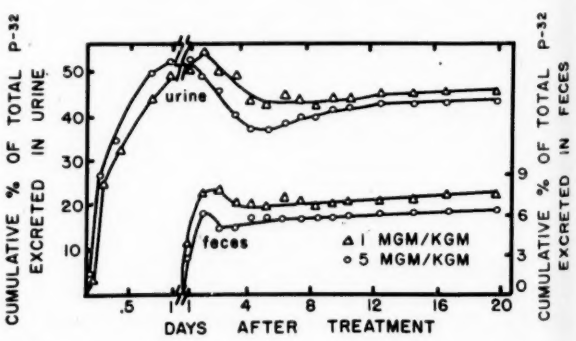

Fig. 3.—Relative excretion rate of radioactive technical Hercules AC-528 by female rats treated orally with 1 or 5 mg. per kg. per day for 10 consecutive days. (Averages of two replicates of two rats each. Cumulative percentages based on total intake versus total excreted in each 24-hour period except during 11- to 20-day period based on 48-hour intervals.)

Table 3.—Tissue residues in female rats 48 hours after oral administration of various radioactive Hercules AC-528 components at 25 mg. per kg.

	P.P.M. BASED ON TOTAL P^{32}[a]							
	Fraction I-P^{32}		*Trans*		*Cis*[b]		Dioxene	
TISSUE	Total	n-hexane Soluble	Total	*n*-hexane Soluble	Total	n-hexane Soluble	Total	*n*-hexane Soluble
Liver	0.73±0.18	<0.01	2.42±0.09	<0.01	4.10	<0.01	1.37±0.14	0.003
Fat	0.02±0.01	<0.01	1.12±0.19	0.68	0.46	0.34	0.30±0.03	0.02
Kidney	0.17±0.05	<0.01	1.16±0.10	0.14	1.37	0.08	0.41±0.04	0.013
Muscle	0.0	<0.01	0.31±0.04	0.05	0.56	0.05	0.08±0.02	0.013
Brain	0.01±0.01	<0.01	0.05±0.02	<0.01	0.18	<0.01	0.01±0.01	<0.01
Heart	0.01±0.01	<0.01	0.02±0.04	<0.01	0.39	<0.01	0.12±0.03	<0.01

[a] Average of two replicates of two rats each. Variation of data is indicated as standard error.
[b] Average from two rats.

urine within 48 hours was similar for the *cis*, *trans*, dioxene and I-P^{32} fractions (36 to 45%), but a higher per cent of fraction I-P^{32} (25%) was eliminated in the feces than the other fractions (5 to 6%). From the percentage of *n*-hexane-soluble radioactivity recovered from the feces (table 5) or from the urine (table 4), the *cis* and *trans* isomers were more stable in rats than the dioxene derivative or fraction I-P^{32}. Chromatography on the partition column of the *n*-hexane-soluble radioactivity recovered from the feces of rats treated with each fraction showed that 1% or less of the radioactivity was eluted with acetonitrile as more polar metabolites (table 5).

The hydrolytic products excreted in the urine during the first 12 hours after treatment at 15 mg. per kg. were studied with ion exchange chromatographic techniques (Plapp & Casida 1958). Diethyl phosphoric, phosphorothioic and phosphorodithioic acids appeared in the urine from each of the radioactive components (table 4). The *cis* and *trans* isomers yielded more diethyl phosphoric acid than the other fractions. The diethyl phosphorodithioic acid could only arise from direct P—S+C cleavage. The diethyl phosphorothioic acid may have been formed by oxidation to the phosphorothiolate and then cleavage of the P—S+C bond, or by direct hydrolysis of the P+S—C group of the phosphorodithioate, or through further metabolism of the diethyl phosphorodithioic acid. The diethyl phosphoric acid could have originated from oxidation to the phosphorothiolate followed by hydrolysis of the P+S—C group or through metabolism of the diethyl phosphorothioic or dithioic acids.

Table 4.—Hydrolysis products of radioactive Hercules AC-528 fractions from human plasma and rat urine.

HERCULES AC-528 FRACTION	PER CENT HYDROLYZED[a]	PER CENT OF TOTAL HYDROLYSIS PRODUCTS AS:[b] $(C_2H_5O)_2P(O)OH$	$(C_2H_5O)_2P(S)OH$	$(C_2H_5O)_2P(S)SH$
Human plasma[c]				
Fraction I-P^{32}	98.6	0	22.4	77.6
Trans	82.1	0	83.2	16.8
Cis	82.8	0	77.9	22.1
Dioxene	75.0	0	91.0	9.0
Rat urine[d]				
Fraction I-P^{32}	99.6	9.6	56.0	34.4
Trans	94.7	10.0	87.5	2.5
Cis	96.2	11.5	79.0	9.5
Dioxene	99.4	5.3	45.4	49.3

[a] Per cent hydrolysis represents the proportion of P^{32} remaining in the plasma fluid or rat urine after six extractions with equal volumes of *n*-hexane.
[b] Per cent of total P^{32} from one determination using an anion exchange column (Plapp & Casida 1958) with identifications based on chromatography with known non-radioactive carrier compounds.
[c] Hercules AC-528 fractions incubated at 100 p.p.m. with 25 ml. human plasma (pH 7.2) for 24 hours.
[d] Urine collected for first 12 hours after oral administration to two female rats of Hercules AC-528 fractions at 15 mg. per kg.

Studies on Possible Accumulation of Hercules AC-528 in the Fat of Rats.—When several radioactive Hercules AC-528 fractions were fed to rats at 25 mg. per kg. and the rats sacrificed after 48 hours, the fat from rats treated with the *cis* and *trans* isomers was the only tissue having appreciable amounts of *n*-hexane-soluble radioactivity. This *n*-hexane-soluble radioactivity chromatographed entirely as the isomer administered.

The possible accumulation of Hercules AC-528 components in the fat was studied with rats fed for several days with 5 or 10 μg. per kg. of technical Hercules AC-528 or with 2.5 mg. per kg. of the *cis* isomer (table 2). The fat samples were extracted and analyzed by Dr. C. L. Dunn of the Hercules Powder Co. Fat samples averaging 5.5 gm. each (2 to 8 gm. range) were ground in a mortar and pestle with twice their weight of anhydrous sodium sulfate. About 50 ml. of *n*-hexane was added and the mixture stored in a glass jar with occasional shaking for 24 to 48 hours. The *n*-hexane solution was extracted with acetonitrile, and the acetonitrile solution diluted with water and the Hercules AC-528 recovered by extracting it with *n*-hexane. Further clean-up and analysis was by the residue procedure described by Dunn (1958). The final isolation of the glyoxal derivative employed a 1-mm. capillary tube filled with alumina to more easily separate the 2 to 10 μg. quantities of glyoxal 2,4-dinitrophenylosazone. All analytical results were corrected for an average background of 3.0 μg. apparent pure Hercules AC-528 (range of 2.4 to 3.7 μg. for eight rats). Fat analyses yielding less than 0.2 p.p.m. apparent Hercules AC-528 may not be real but due to the result of variation in background. A small but definite amount of Hercules AC-528 was recovered from the fat by colorimetric analysis .The maximum level, 0.6 p.p.m., was reached with the females within 3 days of feeding and this level held constant through the 21 days of feeding. The 0.6 p.p.m. maximum resulted with rats which were assayed 1 day after feeding of technical Hercules AC-528 had been discontinued. When this interval was increased to 10 days after Hercules AC-528 feeding was stopped, residues of Hercules AC-

Table 5.—Metabolism of radioactive Hercules AC-528 fractions by several biological systems.

Biological System	Hours Exposure	Per Cent Present as:[a]											
		Fraction I-P^{32}			*Trans*			*Cis*			Dioxene		
		Unch.	Met.	Hydro.	Unch.	Met.	Hydro.	Unch.	Met.	Hydro.	Unch.	Met.	Hydro.
Cockroaches (*in vivo*)	0	100.0	0.0	0.0	99.9	0.1	0.0	100	0.0	0.0	99.5	0.5	0.0
Cockroaches	2	54.1	1.2	44.7	73.1	0.7	26.2	81.0	1.4	17.6	58.3	3.3	38.4
Human plasma (*in vitro*)	24	1.4	0.0[b]	98.6	17.9	0.0	82.1	17.2	0.0	82.8	25.0	0.0[b]	75.0
Liver slices (*in vitro*)	11	14.0	0.5[b]	85.5	60.8	0.3	38.9	58.4	0.6	41.0	20.6	1.5[b]	77.9
Liver slices	4	2.5	1.3[b]	96.2	48.8	0.6	50.6	48.1	0.7	51.2	22.3	1.2[b]	76.5
Rat fat (*in vivo*)	48	—	—	—	60.7	0.0	39.3	73.9	0.0	26.1	—	—	—
Rat feces (*in vivo*)	48	3.0	0.01	97.0	51.0	0.4	48.6	51.2	1.2	47.6	14.6	0.2	85.2

[a] Unch. = unchanged Hercules AC-528 fraction; Met. = non-hydrolyzed metabolite(s) portion; Hydro. = hydrolysis products. Unchanged and non-hydrolyzed metabolite percentages based on chromatography on celite with known non-radioactive carrier compounds. Hydrolyzed products represent the proportion of P^{32} remaining in the aqueous medium after six extractions with *n*-hexane or chloroform.

[b] Based on methanol eluate from alumina columns after preliminary elution with benzene.

528 were no longer detectable in the fat. The apparent Hercules AC-528 in the fat was not increased by increasing the daily dose from 5 to 10 mg. per kg. for a 7-day feeding period. Residues resulting with the *cis* isomer fed daily at 2.5 mg. per kg. for 7 days were below the sensitivity limit of the analytical method.

Studies with Hercules AC-528-P^{32} in Cockroaches.—Insect metabolism of Hercules AC-528-P^{32} components was studied in the adult female American cockroach, *Periplaneta americana* (L.). Each radiolabeled material in acetone was applied to the pronotum at 200 μg. per gm. of insect. After 2 hours absorption and metabolism time, the cockroaches were surface-washed with acetone to remove unabsorbed or excreted insecticide and then macerated in 50 ml. water in a Waring Blendor. Particulate materials were removed by filtration through cheesecloth and the aqueous filtrate was extracted eight to 10 times with equal volumes of chloroform. The radioactive materials which could not be removed from the aqueous filtrate by these repeated chloroform extractions along with the phosphorus-32 bound on the particulate fraction were regarded as ionized hydrolysis products (table 5). The combined chloroform extracts were dried with anhydrous sodium sulfate, the solvent removed and the remaining radioactive residue chromatographed on celite with carrier non-radioactive Hercules AC-528. Properties of these metabolites were further studied by determining changes in partition distribution of the radioactivity between *n*-hexane, acetonitrile and water in volume ratios of 2:1:1, and their chromatographic behavior on an aluminum oxide column. Recovery samples were made by adding an equivalent amount of radiolabeled material in chloroform to similar cockroach macerates and subjecting these "zero time" roaches to an identical extraction and chromatographic procedure.

The *cis* and *trans* isomers were much more stable to hydrolysis than the dioxene derivative or fraction I-P^{32} (table 5). The percentage of "unchanged" materials represents that portion of the radioactivity chromatographing on celite in an identical manner to the known non-radioactive component and the "metabolized" materials were eluted from the columns with acetonitrile and were not present from control cockroaches handled in the same manner. The metabolite(s) were more polar than the parent fractions. When the metabolites were partitioned between *n*-hexane, acetonitrile and water, about 80% of the radioactivity remained in the water plus acetonitrile phase, whereas the original Hercules AC-528 components *per se* partitioned about 90% into *n*-hexane. The behavior of the metabolite(s) was also different on alumina columns from that of the parent fractions. The unmetabolized fractions of Hercules AC-528 were quantitatively eluted from alumina columns with benzene. Benzene was not effective in removing the metabolite(s) from alumina but they could be eluted with methanol.

In vitro Studies.—The *in vitro* metabolism of radioactive Hercules AC-528 components by rat liver slices was studied using the general procedure of DuBois *et al.* (1950). About 300 mg. of fresh liver slices were added to Krebs-Ringer phosphate buffer at pH 7.2 containing 0.02 molar radiolabeled phosphorodithioate and 0.1% Triton X-155. The Warburg flasks were gassed with oxygen and incubated at 38° C. for 1 or 4 hours. The flask contents were extracted and fractionated in the same manner as previously described for cockroaches. The *cis* and *trans* Hercules AC-528 were more stable to hydrolysis than the dioxene of fraction I-P^{32} derivatives (table 5). Rat liver slices were no more efficient than the *in vivo* systems in producing the more polar metabolite(s) eluted with acetonitrile from celite. The solubility properties and chromatographic behavior on alumina columns of the rat liver metabolite(s) were the same as those described for the cockroaches metabolite(s).

The rate of hydrolysis of these radiolabeled phosphorodithioates by human plasma was also investigated. The compounds were incubated at 100 p.p.m. with human plasma at pH 7.2 and 37° C. for 0, 0.25, 0.5, 1, 2, 4 and 24 hours. *N*-hexane/water partitioning was used to determine the extent of hydrolysis (method of Arthur & Casida 1957). The initial hydrolysis rate was least for the dioxene derivative, about 10 to 20 times faster for the *cis* and *trans* isomers, and 50 times more rapid for fraction I-P^{32}. As the hydrolysis of the *cis* and *trans* isomers approached 50%, the rate greatly diminished. No such

change in hydrolysis rate was observed on incubation of these isomers with alkali. No dioxene derivative was formed on hydrolysis of *cis* and *trans* Hercules AC-528 by plasma for 24 hours at 37° C. (table 5) and so the change in rate was not due to the formation of the dioxene derivative which was more stable to hydrolysis by plasma. The hydrolysis products from these Hercules AC-528 fractions were determined by precipitating the plasma proteins with acetone, filtering out the proteins, removing the acetone from the aqueous medium by distillation, and chromatographing the aqueous residue on an ion exchange resin (Plapp & Casida 1958). The only hydrolysis products recovered were diethyl phosphorothioic and diethyl phosphorodithioic acids (table 4). It would appear that these acids were formed from cleavage of the P—S+C or P+S—C bonds with no oxidation occurring.

DISCUSSION.—Technical Hercules AC-528 was separated by the partition chromatographic procedure of Diveley *et al.* (1958) into eight different fractions, The major constituents were the *cis* and *trans* isomers of 2,3-*p*-dioxanedithiol *S,S*-bis (*O,O*-diethyl phosphorodithioate). Minor constituents identified were 2-*p*-dioxenethiol *S*-(*O,O*-diethyl phosphorodithioate) and bis(diethoxyphosphinothioyl) disulfide. Two other components are ethyl phosphorothioates without a dioxane or dioxene ring. The final two fractions are composed of mixtures of more polar derivatives.

The *cis* isomeric of Hercules AC-528 was more toxic than the *trans* (isomer configuration according to Diveley *et al.* 1958) to house flies and white rats. The magnitude of difference in toxicity of the geometrical isomers of Hercules AC-528 was not as great as that reported for certain substituted-vinyl phosphate insecticides (Casida 1955, Casida *et al.* 1956). The stability of the *cis* and *trans* isomers of Hercules AC-528 were almost identical in alkali and various biological systems. The *trans* isomers of Phosdrin and other substituted-vinyl phosphates are more persistent than the *cis* isomers under many different conditions (Casida *et al.* 1958, Gatterdam *et al.* 1959).

Acute and sub-acute toxicity of technical Hercules AC-528 to rats cannot be entirely accounted for on the basis of the content of *cis* isomer, even though this isomer is the most toxic of the Hercules AC-528 components. The rate of cholinesterase depression and recovery in rats treated with sub-lethal doses of Hercules AC-528 was similar to that reported for parathion, EPN and Potasan (Frawley *et al.* 1952). Also as with these compounds, Hercules AC-528 was more toxic to female than male rats. The only tissue of rats that was found to have any tendency to accumulate Hercules AC-528 was the fat. Sub-acute feeding studies with technical Hercules AC-528 showed that a low level of fat accumulation occurred, but that the residues in the fat were rapidly lost after feeding was discontinued.

In all the biological systems studied, bis(diethoxyphosphinothioyl) disulfide was the least stable of the radioactive Hercules AC-528 components. The *cis* and *trans* isomers of Hercules AC-528 were always similar in stability and much more stable than 2-*p*-dioxenethiol *S*-(*O,O*-diethyl phosphorodithioate). The single exception was with studies on hydrolysis by human plasma where the dioxene derivative was more stable than the *cis* and *trans* Hercules AC-528 isomers.

O,O-diethyl phosphoric, phosphorothioic and phosphorodithioic acids were recovered as hydrolysis products of the radioactive Hercules AC-528 components from *in vitro* and *in vivo* biological systems. This indicates an enzymatic cleavage of the P—S+C as well as the P+S—C bond along with considerable oxidation before or after hydrolysis.

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The Effect of Certain Insecticides and Fungicides on Fungi Pathogenic to the Spotted Alfalfa Aphid[1]

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ABSTRACT

Laboratory tests have shown that certain insecticides and fungicides are toxic to the vegetative stages of some of the fungi that attack the spotted alfalfa aphid, *Therioaphis maculata* (Buckton). Most susceptible to these materials was *Entomophthora exitialis* Hall & Dunn, the dominant pathogen of the aphid in California. Further tests have indicated that the insecticides do not kill the resting spores of *E. virulenta* H. & D. although germination is retarded to some extent.

The rapid spread of the spotted alfalfa aphid, *Therioaphis maculata* (Buckton), in California was followed within about 2 years by the appearance of several species of entomophthoraceous fungi in the southern part of the state (Hall & Dietrick 1955, Hall & Dunn 1957a, b). Certain of these pathogenic organisms have spread throughout the state by natural and artificial means (Hall & Dunn 1958) and the entomophthoraceous fungi are now considered an important factor in the natural control of the pest in many of the alfalfa-growing areas in California.

Three insecticides, parathion, malathion, and demeton, have been recommended for control of the spotted alfalfa aphid. Of necessity these materials have been used in fields where the parasitic microörganisms are or have been active. Since growers have become conscious of the importance of fostering natural control factors, the question of possible inhibitory effects on the fungi by these insecticides and other materials has been raised on many occasions. In order to answer this question, a laboratory study has been made of the effect of recommended dosages of parathion, malathion, and demeton on the five known species of fungi pathogenic to the spotted alfalfa aphid. Also tested were DDT and Trithion which are other materials that might be used for the control of insect pests on alfalfa. In addition, a number of fungicides, most of which are not recommended for use on alfalfa, were tested to determine their effect on the fungi. The fungicides which were tested at approximately one-third the arbitrary recommended dosage included wettable sulfur, Dithane Z-78, Ferbam, Bordeaux 5–5–50, and Captan. The parasitic fungi subjected to the chemicals were *Entomophthora exitialis* Hall & Dunn, *E. virulenta* H. & D., *E. obscura* H. & D., *E. ignobilis* H. & D., and *E. coronata* (Cost.) Kevorkian.

METHODS.—The method used to test the effect of insecticides involved the use of technical grade materials which are considered at least 90% insecticide and contain no carriers that could be inhibitory to the organisms. The amounts used in the preparations were calculated so that where possible the concentration in the medium would be equal to the concentration in a spray at the recommended dosage. The insecticides were added to sterile Sabouraud Dextrose Agar cooled to a temperature of about 42° C. and the agar-insecticide mixture was shaken vigorously until an even suspension was attained. The mixture then was poured into sterile petri dishes and allowed to solidify. Technical DDT, being almost insoluble in water, was difficult to place into suspension. Since inhibitory solvents could not be used, the DDT crystals were blenderized in water and the resultant mixture was poured into a 13% agar solution to produce the usual (6.5%) agar substrate. The DDT-agar was agitated while cooling to keep the crystals in suspension. In the fungicide tests, it was not necessary to resort to technical grade materials because the commercial products were mixed with inert clay instead of organic solvents and no problem of carrier-inhibition was encountered.

The method used for testing the insecticides and fungicides on the fungus cultures was adapted from Kendrick (1955). The inoculum of each of the five fungus species was prepared by using a flamed cork cutter to cut small plugs (4 mm. diameter) from vigorous pure cultures of the organisms growing on Sabouraud Dextrose Agar in petri dishes. The plugs were lifted with a sterile needle and placed on the insecticide- (or fungicide-) agar plates with the growing mycelium in direct contact with the medium. The tests with each insecticide or fungicide were replicated five times. To assure uniform test conditions, one plug of inoculum of each species of fungus was added to each of the replicates. Untreated control cultures with only the chemical lacking were also set up in replicate. Both the test and control plates were incubated at room temperature (approximately 25° C.) for about 10 days. The plates were examined and the growth of each fungus was rated at the end of that time. Observations were recorded as (1) no growth, (2) poor growth on inoculum only, (3) fair growth on inoculum and substrate, and (4) good growth on inoculum and substrate comparable to the culture growth on untreated agar in the control group. No growth was regarded as indicating that the material was toxic to the fungus. Growth on the inoculum only indicated that the material incorporated in the test agar was inhibitory. Growth on the substrate indicated that the chemical had little or no effect on the fungus.

As a rule, insecticides are used against the spotted alfalfa aphid mainly during periods of the year when environmental conditions are unfavorable for activity by the parasitic fungi. At these times, any insecticidal inhibition would be effected upon the resistant resting spores of the fungi which are present in the rubble in the fields. Recent successes with experimental germination of resistant stages reported by Hall & Halfhill (1958) permitted the qualitative testing of the effect of the various insecticides on the germination of the resting spores of *Entomophthora virulenta*.

Parathion, malathion, demeton, and Trithion (technical materials) were mixed in 100 ml. amounts of water

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with Tween 80 (0.5 ml.) added as a wetting agent. Since technical DDT would not dissolve readily in water, 2.5 gm. of DDT and 25 ml. of acetone were blenderized in 100 ml. of water for 5 minutes, filtered, and the solution heated to 100° C. to evaporate the acetone, leaving an insecticide-water mixture containing a small amount of DDT.

Small quantities of laboratory-produced resting spores of *Entomophthora virulenta* were placed into bottles of the insecticide solutions maintained at room temperature (25° C.). The bottles with spores and insecticides were shaken for 20 minutes with a mechanical shaker to assure thorough wetting of each spore. Then the spore suspensions were filtered and the spores dried; most of the spores had been wet with insecticide for about 6 hours. After drying, the resting spores were resuspended in water and inoculated on petri dish plates of Sabouraud Dextrose Agar and the plates were allowed to incubate at room temperature. Each test was replicated five times. The plates were examined on the third, fifth, sixth, and seventh days and the germination and growth were rated.

RESULTS AND DISCUSSION.—The results of the experiments are presented in tables 1 and 2. It is evident from table 1 that the insecticides in common use against the spotted alfalfa aphid were definitely toxic to the vegetative stages of some of the fungi that attack the aphid. The most widely distributed and best pathogen under natural conditions, *Entomophthora exitialis*, appeared to be the most affected with no survivial noted in the presence of any of the insecticides. On the other hand, *E. coronata* was the least affected by the chemicals and failed to grow only in the presence of Trithion. The most innocuous of the materials tested were parathion and DDT. Demeton and malathion appeared to be more fungicidal in their activities while Trithion was lethal to all species. The fungicides tested varied somewhat in their effect on the entomogenous organisms although the pattern was the same, with *E. exitialis* the most and *E. coronata* the least affected.

Table 1.—Effects of several chemical insecticides and fungicides on the five species of entomophthoraceous fungi parasitic on *Therioaphis maculata*.

MATERIAL	DOSAGE PER 100 ML. OF WATER FOR USE IN TEST AGAR[a]	RATING OF GROWTH OF SPECIES OF *Entomophthora* AFTER A PERIOD OF 10 DAYS[b,c]				
		exitialis	*virulenta*	*obscura*	*ignobilis*	*coronata*
Technical insecticides						
Parathion	0.80 ml.	N	G	P	G	G
Malathion	1.50 ml.	N	N	N	N	G
Demeton	0.24 ml.	N	N	N	N	P
Trithion	1.04 ml.	N	N	N	N	N
DDT	2.50 gm.	N	G	N	N	G
Fungicides						
Wettable sulfur	0.49 gm.	P	G	P	G	G
Dithane Z-78	0.25 gm.	N	G	N	F	G
Ferbam	0.25 gm.	N	N	P	P	G
Bordeaux 5-5-50	2.41 gm.	N	N	P	N	P
Captan	0.25 gm.	N	N	N	F	G
Untreated control	None	G	G	G	G	G

[a] Equivalent to the following amounts: Insecticides (oz. per 7 gallons)—parathion, 8; malathion, 16; demeton, 2; Trithion, 12; and DDT, 24. Fungicides (lbs. per 100 gallons)—wettable sulfur, 4; Dithane Z-78, 2; Ferbam, 2; Bordeaux 5-5-50, 20; and Captan, 2.

[b] Each test replicated five times.

[c] Growth rate symbols: N=no growth, P=growth on inoculum only, F=fair growth on inoculum and substrate, G=good growth on inoculum and substrate comparable to growth on untreated agar in the control group.

Table 2.—The effect of several insecticides on the germination and growth of true resting spores of the parasitic fungus, *Entomophthora virulenta*..

MATERIAL	DOSAGE OF TECHNICAL MATERIAL PER 100 ML. OF WATER	RATING OF THE GERMINATION AND GROWTH AFTER TREATMENT[a,b] (DAYS)			
		3	5	6	7
Parathion	0.80 ml.	N	N	P	G
Malathion	1.50 ml.	N	N	P	G
Trithion	1.04 ml.	N	P	F	G
Demeton	0.24 ml.	N	F	F	G
DDT	2.50 gm.	N	F	F	G
Untreated control	—	G	G	G	G

[a] Each test was replicated five times.

[b] Germination and growth rate symbols: N=none, P=poor, F=fair, G=good.

Since the dominant pathogen of the spotted alfalfa aphid, *Entomophthora exitialis*, is so susceptible, it would appear that treatments with any of the insecticides and (or) fungicides tested when vegetative stages of the fungus are present could be adverse to microbial control of the aphid. However, under normal conditions few insecticidal treatments are made during periods when the fungi are active in the field and the fungicides are rarely used at any time. The results presented in table 2 indicate that the insecticides did not kill the resting spores of *E. virulenta*. However, the treatment did retard germination to some extent although the growth that followed was not affected. Since the soaking treatment with insecticides was severe, it is reasonable to assume that the resting spores of *E. virulenta* should be able to maintain their viability in the field despite repeated insecticidal applications. If the resting spores of the other species, especially *E. exitalis*, are affected in a similar fashion, it is quite possible that the insecticidal programs recommended for the suppression of the spotted alfalfa aphid will not have a harmful effect on the survival of the fungi that attack the spotted alfalfa aphid, thus permitting their reappearance when environmental conditions are favorable.

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The Germination of Resting Spores of *Entomophthora virulenta*[1] Hall and Dunn[2]

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ABSTRACT

Laboratory tests have shown that about 2 to 5% of the resting spores of *Entomophthora virulenta* from pure cultures are ready to germinate when removed from the dry state and placed on artificial media. The germinating spores do not need the influence of chitin-splitting microörganisms or a lengthy period in a moist environment in order to start their growth, but they are responsive to high humidity conditions since a progressive increase in germination was noted as the period that the spores were soaked was increased. Considerable germination was noted following 10-minute exposures to temperatures up to 93° C. while no growth occurred after a 96-hour exposure to a temperature of 85° C.

Up to the present time very little has been learned about the activities of the resting stages of entomophthoraceous fungi. With no experimental data, pioneer workers had to be content with making speculations about the function of the resistive bodies. It is a general belief that they furnish the fungi with a means of withstanding adverse conditions for periods of time, or of hibernating for up to one or more seasons.

The terms "chlamydospores" and "resting spores" are used often to denote the special spores that are formed by the entomophthoraceous fungi to withstand unfavorable environmental conditions. Since there are two general types of resistant spores produced by the fungus species in this group, the broad usage of these terms has resulted in confusion. The following differences between the categories of spores are noted in order to give a clarification of the type that was utilized in the study on which this report is based. Chlamydospores are formed by the hyphal bodies of some species by the development of heavy single walls of variable thickness when conditions of temperature and moisture are not favorable. On the return of a favorable environment, these chlamydospores germinate readily and proceed with the formation of conidiophores. The resting spores are generally formed internally and have thick, triple-layered walls. The outer wall is usually smooth although in some species the covering may be characterized by spines, knob-like projections, or furrows. When mature, the spores are usually spherical and in general they are large in size. They may be formed by an asexual budding from a hyphal body or cell, wherein they are known as "azygospores," or they may result from a true sexual conjugation between a pair of hyphal bodies or cells in which case they are called "zygospores."[3] Typical resting spores are pictured in figure 1.

According to Thaxter (1888), claims of germination of resting spores were made by Nowakowski, who stated that spores placed in water in autumn germinated during the following spring. However, Thaxter questioned the accuracy of this report, since he never observed germination even though spores were kept in water for as long as

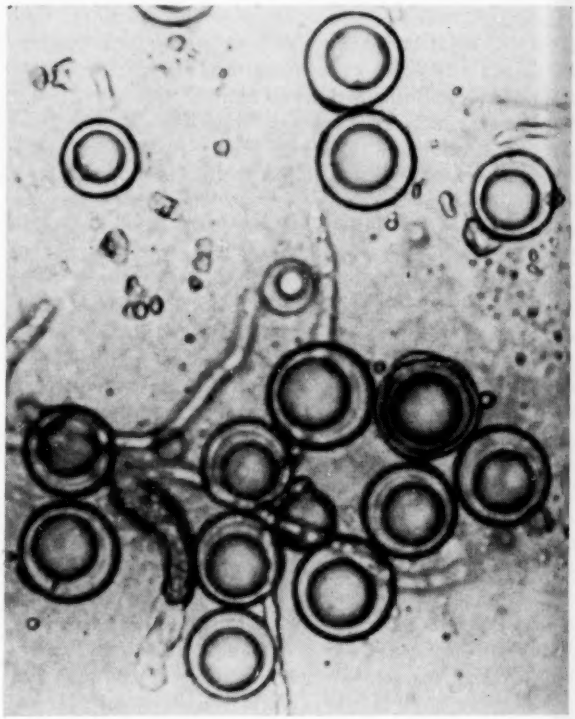

Fig. 1.—Resting spores of *Entomophthora virulenta*.

3 months. Gilliatt (1925) recorded germination of the resting spores of *Entomophthora sphaerosperma* Fresenius in water 16 days after their suspension in Van Tieghem cells. Sawyer (1931), who failed in every attempt to bring about germination of the azygospores of the same fungus although he tried freezing, drying, heating, treatment with acid and simple suspension in water, suggested that the illustrations used by Gilliatt indicate that he may have succeeded in germinating hyphal bodies rather than true resting spores.

Schweizer (1947) reported that resting spores of *E. muscae* Cohn were stimulated to germinate by bacteria that break down chitin. He suggested that pure cultures of entomophthoraceous fungi also could be obtained by subjecting resting spores to a bacteria-free filtrate from a concentrated culture of chitin-destroying bacteria or the enzymes retained in the fluids of a cold-sterilized bacterial culture.

[1] Entomophthorales.

[2] This investigation was supported by Grant E-1333 from the U. S. Public Health Service. Paper No. 1064, University of California Citrus Experiment Station, Riverside. Accepted for publication July 16, 1958.

[3] Although the fungus, *Entomophthora virulenta* H. and D., does not form chlamydospores on artificial media, it does produce an abundance of resting spores. Most of these are azygospores although a few zygospores have been noted.

Recent studies on the use of microorganisms in the biological control of the spotted alfalfa aphid, *Therioaphis maculata* (Buckton), in California resulted in the isolation and cultivation on artificial media of five species of Entomophthorales (Hall & Dunn 1957 a and b). During the course of investigations of the growth characteristics of these fungi on natural and synthetic media, interest in the germination of resting spores was aroused when the empty cells of germinated spores were noted in a 35-day-old pure culture of *Entomophthora virulenta* on Sabouraud maltose agar. Although this phenomenon was not observed again in the many examinations of cultures of varying ages of all fungus species under study, it gave the indication that the resting spores may be capable of germination when conditions become favorable without the lengthy time interval suggested by some early workers or the complex action by chitin-splitting organisms as formulated by Schweizer.

MATERIALS AND METHODS.—The fungus, *E. virulenta* (resting spores of which appeared to have germinated in an old culture) was selected for study in controlled germination tests. Its cultural characteristics made it an ideal test organism since it produced great numbers of thick-walled azygospores quickly on synthetic media, yet did not form chlamydospores.

The spores used in the initial germination tests were obtained from cultures grown on 10% Sabouraud dextrose agar in 6-liter Povitsky bottles in a manner similar to that described by Steinhaus (1951) for the production of the spore-forming bacterium, *Bacillus thuringiensis* Berliner. Each bottle was inoculated by aseptically introducing a slurry of fungus material prepared by blenderizing 7-day-old pure cultures of *E. virulenta* in sterile water. The fungus grew rapidly on the surface of the media forming dense colonies. After 14 days, when the production of resting spores had almost ceased, the material was carefully scraped from each culture bottle, removing as little of the agar as possible, and the harvested spores were spread out to dry in an open pan. The resultant brittle spore mass was triturated with mortar and pestle into a powder which was sieved through an organdy cloth screen to remove the large particles. The final material consisted of many clumps of true resting spores along with a limited amount of extraneous material. Every attempt to break up the clumps by soaking, shaking, blenderizing, treatment with acids or the addition of various wetting agents ended in failure. It was apparent that the mucilaginous material on the surface of the spores is extremely water-resistant when dry and cannot be dissolved by methods that do not result in the destruction of the spores.

To obtain essentially clump-free spore material for subsequent quantitative germination experiments, freshly produced spores were suspended in distilled water, shaken for 30 minutes, and then concentrated by low-speed centrifugation. After the supernatant was poured off, the spore material was washed and centrifuged several times until the supernatant remained clear. Following a final decanting, the spore material was placed in a drying oven at 32° C. to evaporate the remaining water. The product, although not completely free of conglomerations of spores, was much improved over that from the initial harvest. Most of the spores were free when the dried material was resuspended in water. The limited number of aggregations that remained were found to contain, in most instances, less spores per clump than were present in the first product.

EXPERIMENTAL RESULTS.—The first attempt to germinate resting spores of *E. virulenta* was made on a special peptone-dextrose agar containing rose bengal and streptomycin that was developed by Martin (1950) for use in making colony counts of soil fungi. Five petri dishes containing the culture medium were used in the first test. Inoculation was accomplished by dusting a small quantity of the dry spore material into each dish. The dishes were incubated at room temperature (about 25° C.). Within 72 hours numerous small fungus colonies were noted on the surface of the medium. These colonies were examined microscopically and the fungus was identified as *E. virulenta*.

Following this initial success at germination, resting spores were dusted on Sabouraud dextrose agar in petri dishes and many colonies of *E. virulenta* developed. Bacterial contamination was observed in some of the test dishes since no antibiotic was incorporated in the culture medium to inhibit growth of the bacteria present in the air-dried resting spore material. Subsequent germination tests on Sabouraud dextrose agar containing streptomycin resulted in the formation of numerous colonies of *E. virulenta* in each of the replicated dishes, with no bacterial contamination to suppress the fungus growth. Transfers of some of the small colonies to Sabouraud dextrose agar slants resulted in the development of pure cultures of the fungus. Using the same spore materials as the inoculum, the duplicability of these germination tests was verified in additional replicated experiments (see fig. 2).

Considerable effort was made to determine the number of resting spores of the fungus per gram of dry spore material. Numerous spore counts were made in known dilutions of the clump-free material, utilizing a haemocytometer and a 10-lambda pipette. The results were somewhat erratic, but the count of about 1.36×10^8 spores per gram of dry material was obtained most often with both counting methods, and it was considered as the standard for the batch of spore material used in the experiments.

The first quantitative germination tests of resting spores of *E. virulenta* were conducted by inoculating petri dishes containing Sabouraud dextrose agar with streptomycin with known numbers of spores in sterile water. Germination of spores and resultant colony development were determined over a period of up to 4 days. This time limit was instituted because the colonies grew rapidly and quickly produced conidia which were expelled in great numbers; the many secondary colonies forming from the germinating conidia made impossible within a short period of time any accurate counts of resting spore germination. The results of the first series of quantitative spore germination tests are presented in table 1.

The results presented in table 1 show that a small number of resting spores of *E. virulenta* are capable of germinating quickly when placed in a suitable environment. It is evident that about 2 to 5% of the resting spores may be ready to germinate almost immediately when removed from the dry state and placed on a satisfactory artificial medium. These spores apparently do not need the ex-

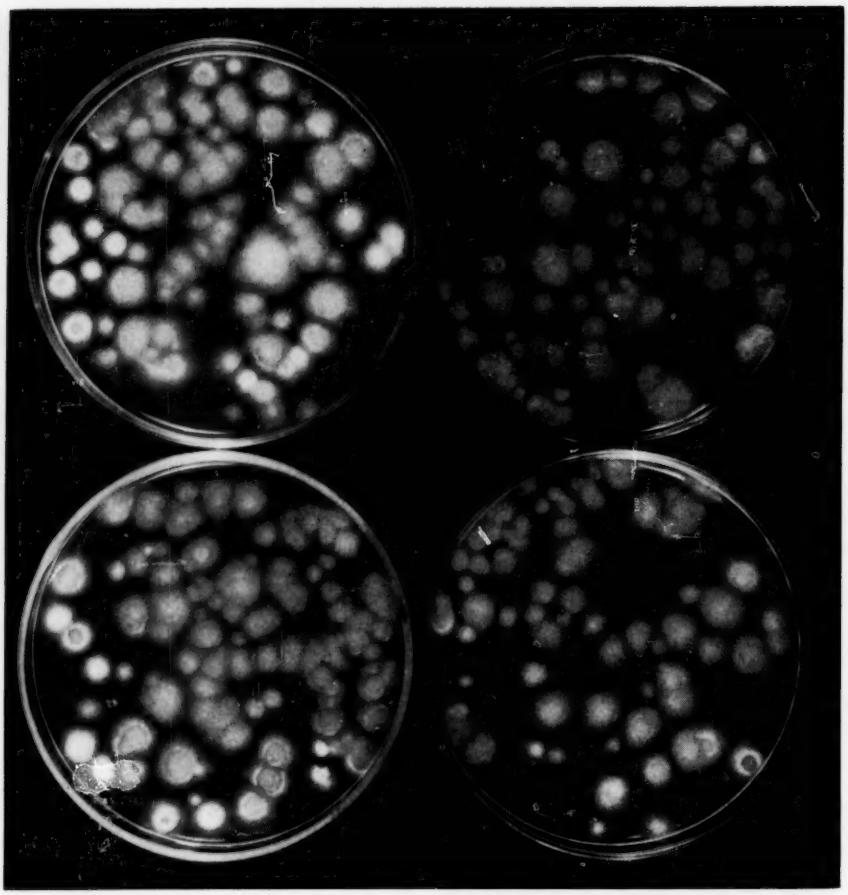

Fig. 2.—Colonies of *Entomophthora virulenta* resulting from the germination of resting spores on Sabouraud dextrose agar.

ternal stimulation of a chitin-splitting enzyme as claimed by Schweizer (1947), or a lengthy period in a moist environment in order to start their growth. Some of them must be ready for almost immediate germination since colony counts at the end of 2 days appeared to be about the same as those after 4 days.

Since entomophthoraceous fungi are known to be more active in nature during periods of high humidity, there has been speculation over the year that the resting spores may be induced to germinate by the soaking action of rain, dew, or irrigation water. Evidence to support this idea was brought forth by Gilliattt (1925) in his report on attempts to germinate resting spores of *Entomophthora sphaerosperma* in both nonsterile tap and sterilized water. He observed the germination of resting spores in hanging drops of tap water at the end of a 16-day period and followed the growing process for several days. Gilliatt concluded that the spores of *E. sphaerosperma* evidently need a considerable period of moisture for germination, at least under the conditions of his experiment.

An experiment was set up to determine the effects of prolonged soaking in water on the germination of resting spores of *E. virulenta*. Preliminary tests using sterilized tap water and water with streptomycin indicated that the antibiotic would be a necessary additive during the soaking period to prevent the build-up of bacterial contamination on the culture media while the resting spores were germinating. Heavy contamination was encountered when spores were soaked in water and then placed on a medium with or without streptomycin while little contamination occurred when spores soaked in a water-

Table 1.—Percentage of resting spores of *Entomophthora virulenta* germinating on Sabouraud dextrose agar over a period of up to 4 days.

Test No.	Period of Germination (days)	Spores Applied per Plates	No. of Plates	Average No. Germinating Resting Spores/ Plate	Per Cent Germinating Resting Spores[a]
1	2	680	2	30.5	4.5
		68	2	4.0	5.8
2	2	1360	2	31.0	2.3
		136	2	7.0	5.1
3	4	680	8	31.3	4.6
		68	8	4.0	5.8
4	4	680	8	18.0	2.6
		68	8	3.5	5.1

[a] Based on a spore count of 136 million spores per gram of dry spore powder.

Table 2.—**Percentage of resting spores of *Entomophthora virulenta* germinating on Sabouraud dextrose agar after soaking in water fortified with streptomycin.**

Length of Soaking Period (hours)	Resting Spores Applied per Plate	No. of Plates	Average No. Germinating Resting Spores/ Plate[a]	Per Cent Germinating Resting Spores
16	680	6	25.7	3.8
32	680	6	54.3	8.0
56	680	6	98.5	14.5
Control (nonsoaked)	680	8	21.1	3.1

[a] Test plates were counted after two days. Control plates were counted after three days.

streptomycin bath were cultured on an unfortified medium.

The stock solution of the antibiotic used in this study contained 6670 micrograms of streptomycin per ml. of sterile distilled water. A 1:100 dilution of the spores was prepared by mixing 0.05 mg. of dry spores with 9 ml. of sterile water and 1 ml. of antibiotic solution. The spores were soaked for periods of 16, 32, and 56 hours, the latter time being set as the maximum because many spores were germinating in the bath and growth was becoming visible. After each soaking period, 1 ml. amounts of the spore suspension were placed in petri dishes containing Sabouraud dextrose agar, and the dishes were incubated at room temperature (25° C.). The fungus colonies were counted at the end of 2 days in the test dishes and after 3 days in the control dishes. The results are presented in table 2.

It is evident that there is a progressive increase in the number of spores that germinate as the period of soaking is increased and at the 56-hour limit of the test the increase in germination was almost 500%. At that, only 14.5% of the spores did germinate. It is possible that there would be a continuation of the progressive pattern of germination if the spores could be subjected to longer periods of soaking.

To determine the effect of temperature on spore germination, dry resting spores of *E. virulenta* were kept at 66° C. for periods of 5, 8, 24, and 96 hours. Five milligrams of heat-treated spores from each period were suspended in 10 ml. of water immediately after removal from the incubator and 1 ml. of the suspension (68,000 spores) was placed in each petri dish containing Sabouraud dextrose agar forified with streptomycin. The results are presented in table 3.

When compared with the minimum 2.3% germination of untreated resting spores presented in table 1, there was a marked drop off in germination when the spores were subjected to a temperature of 66° C. for as short a time as 5 hours. Although the decrease in germination was only slight between 8 and 96 hours, there was a definite lag noted in the speed of germination. In the 5-, 8-, and 24-hour groups, the first germinations were observed after 2 days with maximum germination noted 1 to 2 days later. In the 96-hour group, germinations were detected on the fourth day with the maximum number of new colonies appearing at the end of 6 days.

Table 3.—**Percentages of resting spores of *Entomophthora virulenta* germinating on Sabouraud dextrose agar after exposure for varied periods to a dry heat of 66° C.**

Hours at 66° C.	No. of Plates	Period of First Germination (days)	Period of Total Germination (days)	No. of Resting Spores Applied per Plate	Average No. Germinating Resting Spores/ Plate	Per Cent Germinating Resting Spores
5	6	2	4	6.8×10^4	76.1	0.11
8	6	2	4	6.8×10^4	47.3	0.07
24	6	2	3	6.8×10^4	52.0	0.08
96	6	4	6	6.8×10^4	38.2	0.06

In subsequent tests on the effect of temperature, dry resting spores were maintained at temperatures of 71° C., 77° C., and 85° C. for 96 hours. At the end of this period, the spores were suspended immediately in a water-streptomycin solution. One-milliliter amounts of this spore suspension were placed in each of five petri dishes containing Sabouraud dextrose agar fortified with the antibiotic. The remaining spores were left in the solution to soak for 50 hours and then 1-ml. amounts of the suspension were placed on the culture medium in each of five petri dishes. The dishes in each test group were incubated at room temperature (25° C.). The results are presented in table 4.

Although the number of spores that germinated was small after exposure to a temperature of 71° C. for 96 hours, the results indicate that soaking for a period of 50 hours brought about a six-fold increase in germination. No significant differences between germinations of soaked and unsoaked spores were noted in the group subjected to a temperature of 77° C. and it is apparent that prolonged exposure to a temperature of 85° C. results in loss of viability by the spores.

Another group of resting spores was kept at 121° C. in an autoclave. As soon as this temperature was reached, the autoclave heater was turned off and the chamber was vented. The spores were removed immediately and inoculated into petri dishes containing Sabouraud dextrose agar. At the end of 13 days no germination was observed indicating that the spores had been killed.

A number of tests in which the spores were subjected to high temperatures for a period of 10 minutes were conducted to determine the thermal death point of the resting spores of *E. virulenta*. Considerable germination was noted at temperatures up to 93° C. However, none of the spores displayed viability after a 10-minute exposure to 121° C. indicating a flash death point somewhere below that temperature.

The role of chitin-splitting bacteria on resting spores of entomophthoraceous fungi in nature and in artificial

Table 4.—**Percentages of resting spores of *Entomophthora virulenta* germinating on streptomycin-fortified Sabouraud dextrose agar after exposure to varied temperatures.**

Test Temp. (° C.)	Hours of Exposure	Period of Subsequent Soaking (hours)	No. of Plates	No. Spores Applied per Plate	Total Days Plates Observed	Average No. Spores Germinating per Plate	Per Cent Germination of Resting Spores
71	96	50	5	6.8×10^4	5	62.0	0.091
71	96	0	5	6.8×10^4	7	10.0	0.015
77	96	50	5	6.8×10^4	13	3.6	0.005
77	96	0	5	6.8×10^4	15	3.2	0.005
85	96	50	5	6.8×10^4	20	0.0	0.000
85	96	0	5	6.8×10^4	22	0.0	0.000

Table 5.—The effect of a chitin-splitting bacterium on the germination of resting spores of *Entomophthora virulenta*.[a]

Test No.	Group	Hours in Contact with Chitin-Splitting Bacteria	No. of Replicated Plates[b]	Cumulative Percentage Germination of Resting Spores on Days After Germination				
				1	2	3	4	7
1	control	0	10	2.38	9.96	10.10		
	test	48	10	0.46	3.37	4.16		
2	control	0	10	1.82	11.07	11.26		
	test	48	10	0.10	0.16	0.29	—	0.66
3	control	0	10	—	3.43	—	4.43	
	test	25	9	—	3.39	—	4.34	

[a] Spores of each control group were soaked for similar periods in streptomycin solution.
[b] Number of resting spores inoculated per plate was 680 in each plate.

culture has been discussed by Schweizer (1947) who claimed that this type of stimulation was necessary for the germination of these stages. He reported obtaining almost 100% germination of the resting spores of *E. muscae* after subjecting them to the chitin-splitting action of certain organisms. Since our experiments never had achieved more than 14.5% germination, an effort was made to determine whether or not this type of stimulation could bring about an increase in the number of germinating spores of *E. virulenta*.

Several cultures of different chitin-splitting bacteria were received from D. M. Reynolds, Department of Bacteriology, University of California, Davis, and C. R. Bovell, Division of Life Sciences, University of California, Riverside. At their recommendation, an unidentified organism designated as C-25 was cultivated and used in the tests. In the first two experiments, resting spores were inoculated into nutrient broth containing the chitin-splitting bacterium and the spores were left in contact with the bacterium for 48 hours. The suspension then was diluted with streptomycin solution and known quantities of spores were inoculated into petri dishes containing Sabouraud dextrose agar fortified with the antibiotic. The resting spores of the control groups were soaked for a 48-hour period in a solution of streptomycin and then were set up on culture media. The third experiment was similar to the first two except that the test resting spores remained in contact with the chitin-splitting bacterium for only 25 hours and the spores of the controls were subjected to streptomycin for a like period. The results are presented in table 5.

In comparison with their respective controls, tests 1 and 2 culminated in marked reductions in the number of germinating resting spores. Although both were run under the same conditions, growth of the chitin-splitting bacterium in the culture plates of test 2 despite the treatment with streptomycin no doubt caused the more striking reduction in spore germination and colony formation. The results of test 3 indicate that a 25-hour exposure to chitin-splitting activity neither stimulates nor inhibits spore germination. However, it is evident that a 48-hour treatment with the C-25 isolate used in the tests greatly inhibits germination of the resting spores of *E. virulenta*.

Discussion and Conclusions.—Probably the most controversial work dealing with a study of the germination of resting spores of entomophthoraceous fungi is that of Schweizer (1947) who made a generalization that the azygospores and zygospores of all *Empusa* species, which originate from pure cultures, do not germinate except when conditions are created which correspond to those in nature at the time of germination. According to Schweizer, the resting spores require for their germination special chemical influences which emanate from the chitin-decomposing microörganisms. In order to induce resting spores from pure cultures to germinate in the laboratory, he states that the action of chitin-splitting bacteria during the resting period of the spores is necessary for the spores to become active and germinate.

The recent observation of the remains of germinated resting spores in a pure culture of *Entomophthora virulenta* brought forth the realization that some of the generalizations made by Schweizer for the entire group of entomophthoraceous fungi could be in error. The subsequent findings reported herein tend to bear this out since in some instances they are exactly opposite to the claims of Schweizer. Since our study was limited to only one species, *E. virulenta*, no group-wide generalizations can be made. It is evident, however, that the generalizations put forth by Schweizer should be considered only to concern *Entomophthora* (*Empusa*) *muscae* Cohn, the species with which he worked.

About 2 to 5% of the resting spores of *E. virulenta* from pure cultures are ready to germinate almost immediately when removed from the dry state and placed on suitable heat-sterilized artificial media. The spores that germinate apparently do not need the external chemical influences which emanate from chitin-splitting microörganisms or a lengthy period in a moist environment in order to start their growth. The progressive increase in germination as the period that the spores are exposed to high moisture conditions is increased, indicates that the resting spores of *E. virulenta* may be more responsive to the influence of humidity than are those of *E. sphaerosperma* that were studied by Gilliatt (1925) and Sawyer (1931).

It is evident that high temperatures greatly reduce the number of spores that are able to germinate and that no growth occurs after a 96-hour exposure to a temperature of 85° C. In tests in which the spores were subjected to high temperatures for a period of 10 minutes, considerable germination was noted following treatment at temperatures up to 93° C. None of the spores displayed any viability after a 10-minute exposure to 121° C. indicating a flash death point somewhere below that temperature. The spores also were unable to survive short exposure to steam at a temperature of 121° C.

The series of successful germination studies in pure cultures without external chemical influences followed by the experiments with a chitin-splitting bacterium indicate that the resting spores of *E. virulenta* did not require the action of chitin-splitting microörganisms for germination. In fact, extended exposure to such chemical activity greatly inhibited germination of the resting spores.

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Early Season Cotton Thrips Control in the Yuma, Arizona Area[1]

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ABSTRACT

In annual tests from 1954 through 1957 for the control of thrips, mainly *Frankliniella occidentalis* (Perg.), on seedling cotton near Yuma, Arizona, 11 insecticides, including chlorinated hydrocarbons, phosphorus compounds and systemic insecticides were tested one or more times in foliage applications or seed treatments. In no case was there a significant increase in yields of seed cotton as compared with untreated plots, although thrips populations were at least temporarily reduced in numerous cases. Thrips populations were relatively light, except in 1954, in this area of comparatively low altitude.

Early season control of thrips on cotton is recommended in Arizona in areas of higher altitude (over 1500 feet) and with a relatively shorter growing season (Roney 1958) on the basis of experimental work by Kauffman & Stevenson (1953 plus unpublished work).

This is a report of field experiments from 1954 through 1957, conducted near Yuma, Arizona in an area of low altitude (less than 300 feet) and an extended growing season, to measure possible effects of early season thrips control on cotton yields. Although thrips have seldom been considered to be present in highly injurious numbers on seedling cotton in this area, there remained the question of whether thrips damage might not be of more importance than was commonly realized. The predominant species was *Frankliniella occidentalis* (Perg.). Infestations in seedling cotton were higher in 1954 than in the three succeeding seasons.

Each year treatments were applied to plots of 0.8 acre, replicated four times. Plots were sown with delinted cotton seed at the rate of 21 pounds per acre, except for plots sown with Thimet-treated seed, where a lower rate was applied because of the slower movement of this seed through the hopper of the planter. Infestations were measured by shaking seedling cotton plants over a black surface and counting the numbers of thrips present. After the seedling stage the plants were treated each season with various recommended insecticides as required for the control of other insect pests.

TESTS IN 1954.—The field tests in Experiment 1 were conducted in cotton adjacent to an alfalfa field from which the thrips were migrating. Some thrips injury was noted on the small cotton plants at the time the first spray treatments were applied. The insecticides were applied with a low volume, tractor-mounted sprayer using 3 gallons of water per acre. The poor control obtained with dieldrin, heptachlor and toxaphene indicated that the thrips were apparently becoming resistant to the chlorinated hydrocarbon insecticides in the same manner as onion thrips (Richardson & Wene 1956). (Table 1). The data also show that two applications of the various insecticides did not give increased yields of seed cotton. Malathion was very effective in decreasing the thrips population and yet this control was not reflected in an increased yield, probably due to the long growing season which allowed the cotton plant to compensate for any early season injury.

Table 1.—Results of insecticidal sprays for control of thrips on seedling cotton. 1954.

Toxicant and Pounds per Acre	No. of Thrips per 40 Plants on Days After First Treatment Shown				Pounds of Seed Cotton per Acre
	2	15	17	32	
	—	—	2[a]	15[a]	
Toxaphene, 0.5	65	119	63	72	3835
Heptachlor, 1.0	256	113	12	184	3836
Dieldrin, 0.75	234	181	61	149	3883
Malathion, 0.75	1	63	3	84	3768
Untreated	374	193	119	201	3838

[a] Days after second treatment.

TESTS IN 1955.—In Experiment 2, results of which are shown in table 2, Thimet and Am. Cyanamid 12008 were added to the seed in a carbon powder formulation. Demeton and schradan in water solution were sprinkled on the seed, which was allowed to dry before planting. The data in table 2 show a low thrips population, with Thimet and 12008 giving the best control 24 days after the plants had emerged. Demeton and schradan, as seed treatments, failed to give effective control. The increases in yield were insignificant. Stand counts showed 32% fewer plants in the Thimet-treated plots and 68% fewer in the 12008-treated plots. Part of the reduction in stand can be attributed to the systemic seed treatment formulation, which affected the planting rate of the seed. The yields in treated plots were larger, although to an in-

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[2] Department of Entomology, University of Arizona Agricultural Experiment Station, Tucson.

Table 2.—Effectiveness of seed treatments for thrips control on seedling cotton. 1955.

Toxicant and Pounds per Acre	Thrips per 40 Seedlings on Days After Emergence Shown				Pounds of Seed Cotton per Acre
	10	17	24	31	
Thimet, 0.8	0	0	9	48	3502
Am. Cyanamid 12008, 0.8	0	0	10	101	3870
Demeton, 0.1	0	63	32	86	3648
Schradan, 0.1	1	34	68	91	3582
Untreated	12	76	85	116	3265

significant extent, than in the untreated plots and it is evident that in later growth the surviving plants in the treated plots were able to compensate for the initial loss in stand.

Tests in 1956.—In Experiment 3, Thimet and Bayer 19639 were applied to the seed in a carbon powder formulation. Chlordane and BHC were applied to the seed as slurries. The data in table 3 show that Thimet and Bayer 19639 gave effective control of thrips for 22 days or more, but that this control was not reflected in any increases of seed cotton. Chlordane and BHC added as slurries gave no thrips control or any increase in yields of seed cotton.

In 1956 bloom, square and yield counts were taken in two experiments where Thimet had been applied to the seed in a powder formulation. The data in table 4 indicates that the Thimet treatment had very little effect on the number of blooms or squares set by the cotton plant. As

Table 3.—Effectiveness of seed treatments for thrips control on seedling cotton. 1956.

Toxicant and Pounds per Acre	Thrips per 40 Seedlings on Days After Emergence Shown				Pounds of Seed Cotton per Acre
	7	15	22	29	
Bayer 19639, 0.5	2	5	15	16	4086
Thimet, 0.5	1	5	16	28	4664
Chlordane, 0.5	9	34	282	57	4036
BHC, 0.5 g.i.	13	28	319	75	4207
Untreated	14	20	131	51	4271

Table 4.—Effect of Thimet seed treatments (0.9 lb. per acre) for thrips control on production of blooms, squares and seed cotton. 1956.

	Experiment 1[a]		Experiment 2[a]	
	Treated	Untreated	Treated	Untreated
Blooms per 400 ft. of row	465	478	419	440
Squares per 400 ft. of row	733	1038	1141	1021
Yield of seed cotton	3960	3858	4035	3970

[a] Blooms and squares were counted 70 days after planting in test 1 and after 67 days in test 2.

also shown in the previous experiments, the Thimet treatment had no significant effect on the amount of seed cotton produced.

Tests in 1957.—In Experiment 5, Thimet and Bayer 19639 were applied to the seed as powder and emulsion formulations, respectively. Thrips counts showed (table 5) very little difference between these treatments although Thimet had more residual effectiveness than Bayer 19639. All seed treatments decreased the yields, but not to a significant extent.

Table 5.—Effect of Thimet seed treatments for thrips control on seedling cotton. 1957.

Toxicant and Pounds per Acre	Thrips per 40 Seedlings on Days After Emergence Shown		Pounds of Seed Cotton per Acre
	19	36	
Thimet (Dust), 0.88	6	65	4436
Thimet (Emulsion), 1.0	1	49	4072
Bayer 19639 (Dust), 1.0	5	123	4336
Bayer 19639 (Emulsion), 1.0	4	114	4121
Check	68	143	4536

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BOOK REVIEWS

(*Continued from page 16*)

Biological Studies on the Armyworm, *Pseudaletia unipuncta* (Haworth), in Tennessee (Lepidoptera: Noctuidae). Samuel G. Breeland. Journal of the Tennessee Academy of Science, Vol. 33, No. 4, pp. 263–347. Oct. 1958.

This is a comprehensive study of the biology of the armyworm based on a doctoral thesis. In the table of contents there is included an introduction, review of literature, methods, synonymy distribution, life history, seasonal studies, natural enemies, and literature.

The first real work on the armyworm was that of C. V. Riley, published in his second Missouri report for 1870. Although there is a large literature on the armyworm, practically all the references have to do with descriptions of outbreaks. Not since the work of Davis & Satterthwait (1916) has there been any fundamental research reported on this important pest. In the words of the author "Although the pioneer work of Riley (1870) gives much valuable information concerning the armyworm, a biological study of the species in the light of present information and under present conditions was urgently needed."

S. Marcovitch

Systemic Insecticides as Seed and Granular Treatments to Prevent Grasshopper Damage to Margins of Winter Wheat[1]

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ABSTRACT

Thimet and Bayer 19639 were tested as seed treatments and as granules in the furrows to protect winter-wheat seedlings from grasshopper damage until they could become established. Greenhouse tests with seed treated with one-half pound of Thimet or 1 pound of Bayer 19639 per 100 pounds of seed gave good kills of adults for 4 weeks. Similar results were obtained from granules in a furrow treatment. Stands were materially reduced by seed treatment but were not affected by granule treatment. In a field test with treated seed the plants reached the three-leaf stage with little injury.

Application of systemic insecticides at seeding time offers a new approach to prevention of fall grasshopper damage to margins of winter wheat. Studies conducted with other plants show that systemic insecticides can be expected to remain in the plant system and retain their toxicity best during periods of slow growth. Except for initial emergence, fall growth of winter wheat is relatively slow, and therefore favorable to the plants' retention of such compounds in amounts sufficiently toxic to kill grasshoppers feeding on the seedlings.

When absorbed by a plant, systemic compounds have been shown to behave in much the same manner regardless of plant species. Ripper *et al.* (1951), in work with schradan-sprayed strawberries, concluded that the absorption and decomposition of systemic compounds depend upon the rate of assimilation and translocation of metabolic substances in the plant, which in turn depends upon the time of year. Heath *et al.* (1952) made similar conclusions and added that the rate of decomposition varies little with species planted at the same time of year. In experiments with alfalfa, Reynolds *et al.* (1957) also showed that the rate of decomposition is a function of the rate of growth.

Plants differ in the quantity of systemic compounds that they can tolerate, particularly from soil or seed treatments, and plant toxicity is closely related to the method of application and dosage used. Ivy *et al.* (1950) obtained much better control of spider mites on cotton with seed soaked in solutions of schradan than when the compound was applied as a dust in attapulgite, but liquid treatment of soil or seed caused reduction in stands and plant injury unless dosages were limited. Tsi (1950) also reported reduced stands and an adverse effect on growth with cotton and bean seeds soaked in solutions of schradan. When activated carbon was used as a carrier, larger dosages were tolerated by cotton seed and seedlings than when the seeds were soaked and the effect of the treatment was found to be more persistent (Ivy 1952). Kantack (1955) noted that systemic insecticides with Carbowax 6000 as a carrier reduced germination of wheat seed but the carrier without the toxicant did not.

Several new systemic compounds show promise of being effective against chewing insects in situations where duration of toxicity and economic considerations allow. Two of these, Thimet and Am. Cyanamid 12008, were effective against overwintering boll weevils on cotton for approximately 3 weeks in tests by Parencia *et al.* (1957a), when seed was treated with activated-charcoal formulations of the compounds. Emergence was reduced in one planting of Thimet-treated seed following a heavy rain on the day after planting. In similar tests the next year (Parencia 1957b) Bayer 19639 was as effective as Thimet but at a higher dosage; however, control of the boll weevil was doubtful. Reynolds *et al.* (1957) conducted extensive tests with Thimet and Bayer 19639 applied to alfalfa, cotton, sugar beet, sorghum, and soybean seed by several methods. The type of treatment and dosage affected germination and stands, and seedlings showed phytotoxic effects. For seed coatings best results were obtained with the charcoal formulation, and granules, either in the seed rows or broadcast, were found safer than seed coatings.

Although there are no data available on residues of systemic insecticides in harvested wheat, studies of their biochemical transformation in plants, insects, and warm-blooded animals, and of their significance in edible plant material indicate that there is little hazard, particularly when applied sufficiently in advance of harvest (Apple *et al.*, Davich *et al.*, March *et al.* 1955, Metcalf *et al.* 1955, 1957; Dahm *et al.* 1956; and Ripper 1957). Alfalfa treated with Thimet and Bayer 19639 at planting time showed no residue problem by the time of first cutting (Reynolds *et al.* 1957). Thimet is rapidly converted to oxidative metabolites in the plant system, which are more potent cholinesterase inhibitors than the parent compound but are finally hydrolyzed to nontoxic substances (American Cyanamid Co. 1957). This early toxicity may present a hazard to livestock allowed to pasture on treated wheat during its early stage of growth.

Losses of winter wheat due to grasshoppers are most severe in areas of strip farming, since this method results in a maximum of marginal area. The degree of damage depends largely upon the dryness of surrounding vegetation and the duration of weather favorable to the insects. As few as two or three grasshoppers to a square yard in marginal habitats can destroy two or more rows of grain by concentrating along the field margin.

Shotwell (1953) stated that in western Kansas 10 migratory grasshoppers per square yard of margin could destroy 2 or 3 rods of seedlings, but would do little damage to plants that had reached the three-leaf stage.

The application of sprays and poison baits must be properly timed to be effective. More than one treatment is often required because of the susceptibility of seedlings to destruction by grasshoppers, the small amount of surface to receive insecticidal deposits, and reinfestation by grasshoppers from surrounding habitats. An effective barrier strip of systemic-treated wheat along threatened margins would give protection without these disadvantages.

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The practical use of a systemic insecticide for grasshopper control in fall wheat depends not only on the degree and duration of its toxicity in the plant system, but on its effect on germination, plant growth, and yield. It must leave no deleterious residue, and if used as a seed treatment, storage for a reasonable period should not reduce germination or plant vigor. Ease and safety in handling are important, and treated seed must flow readily through seeding equipment.

In 1956 and 1957 laboratory and field experiments were conducted with Thimet and Bayer 19639 to determine whether they would persist in wheat seedlings in sufficiently toxic amounts to kill grasshoppers before their feeding destroyed the plants. Observations were also made on the effect of the treatment on germination and emergence of wheat.

LABORATORY TESTS, 1956.—Seed of Genesee spring wheat was treated with an activated-carbon dust containing 50% of Thimet and a 1% solution of methyl cellulose as a sticker. Dosages were ¼ and ½ pound of Thimet per 100 pounds. Seed treated with ¼ pound of carbon per 100 pounds was used as a check. Two-striped grasshoppers (*Melanoplus bivittatus* (Say)) reared in the laboratory were confined in screen cages over wheat plants grown in 6-inch flowerpots, each containing approximately 20 plants. Age of the plants was taken from date of planting. The insects were placed in the cages at intervals, and the dead removed and recorded daily either until the plants were destroyed or a distinct reduction in toxicity became evident. The number introduced at any one time varied from 2 to 6 adults or 4 to 10 nymphs per cage and approximated a continuous infestation of at least one grasshopper per foot of a row of winter wheat.

Table 1 shows that the ½-pound dosage killed all the grasshoppers in 3 days until the plants were 15 days old. The period required for 100% mortality then increased gradually until the experiment was terminated. The ¼-pound dosage had begun to lose its effectiveness by the 10th day, and mortalities were not consistent thereafter.

With the ½-pound dosage there was little damage to the plants up to 27 days after planting (fig. 1). Seedlings were highly toxic to grasshoppers during the critical early stage of growth, and adults were killed after eating small sections of the first leaf. Plants from treated seed that were eaten to ground level recovered with no apparent ill effects. After 27 days the damage increased until at 48 days vegetative growth had been reduced approximately 75%. The ¼-pound dosage did not prevent plant damage. In two out of four tests the plants were reduced to stubs at 22 days and in the other two at 27 days; however, in three of the tests there was sufficient recovery so that the plants could be used for the 48 days of the experiment. All check plants were destroyed by the end of the 15-day test period and new ones had to be introduced. The grasshopper mortality recorded from the untreated plants (table 1) was probably due to starvation.

At the start of the experiment in early March, greenhouse temperatures ranged from 70° to 78° F. As the days became warmer they rose at times to as much as 90° and plants grew vigorously. Data obtained from two groups of test plants of similar age but grown at different temperatures are shown in table 2. The effect of the increased temperature is indicated by the greater height of the plants. All those showing the least growth gave 100%

Table 1.—Mortalities of *Melanoplus bivittatus* caged on wheat plants grown from Thimet-treated seed.

Plants		Grasshoppers		Number of Tests	Per Cent Mortality After (days)			
Age (Days)	Height (Inches)	Stage	Number		3	4	5	10
		One-half pound of Thimet per 100 pounds of seed						
4	1– 1.5	Adult	4	2	100	—		
		Fourth instar	5	1	100	—		
8	2– 3	First-second instars	40	4	100	—		
10	4– 5	Adult	8	4	100	—		
15	5– 7	Adult	8	4	100	—		
22	6– 8	Adult	8	4	75	100		
27	7– 9	Adult	16	4	94	94	100	
38	4– 9	Adult	14	4	79	79	86	100
		Fourth instar	5	1	21	21	29	100
48	5–10	Adult	8	3	13	38	88	100
		One-quarter pound of Thimet per 100 pounds of seed						
4	1– 1.5	Adult	4	2	100	—	—	—
		Fourth instar	5	1	100	—	—	—
8	2– 3	First-second instars	40	4	100	—	—	—
10	4– 5	Adult	8	4	88	100	—	—
15	5– 7	Adult	8	4	75	100	—	—
22	6– 8	Adult	8	4	25	25	63	63
27	7– 9	Adult	8	2	63	63	63	100
	1– 5	Fourth instar	8	2	100	—	—	—
48	5– 8	Adult	2	1	50	50	50	50
		Seed not treated						
4	1– 1.5	Adult	4	2	0	—	—	—
		Fourth instar	5	1	0	—	—	—
8	2– 3	First-second instars	39	4	5	—	—	—
10	4– 5	Adult	4	2	0	0	—	—
15	5– 7	Adult	6	3	0	17	17	17
25	9–13	Adult	4	1	0	0	0	0
28	9–13	Adult	6	1	0	0	0	0

Fig. 1.—Effect of grasshopper feeding on growth of wheat plants from treated seed (1, 3, and 4) compared with an untreated check (2).

mortality in 10 days or less, whereas those that grew fast did not. The increased physiological activity of accelerated growth resulted in a lower concentration of the systemic in the plant system, in accordance with conclusions reached by Ripper *et al.* (1951), Heath *et al.* (1952), and Reynolds *et al.* (1957).

FIELD TEST, SPRING OF 1956.—Seed of Thatcher spring wheat treated with a carbon-methyl cellulose formulation to give 0.44 pound of Thimet per 100 pounds was planted near Harlowton, Montana, on April 19. One 12-foot drill width was planted with treated seed for ¼ mile and then continued ¼ mile further with untreated seed. As soon as seedlings emerged, five cylindrical screen cages, 12 inches high and 5 inches in diameter, were distributed along each ¼-mile test strip. Each cage was placed over as many seedlings as it could cover, and four laboratory-reared adults of the migratory grasshopper (*Melanoplus bilituratus* (Walker)) were placed inside.

After 3 days the average mortality on the treated plants was 55% and on the untreated ones 6.25%. All untreated plants were eaten to the ground, whereas the treated plants were 2 to 3 inches high and without significant damage. After 8 days all the grasshoppers feeding on the treated plants were dead and the plants were 3 to 4 inches high. Mortality in the checks averaged 38% at this time and only a few fragments of seedlings remained in one cage. After 20 days the treated plants were 4 to 5 inches high.

Cool, wet weather delayed germination and growth and materially reduced the toxic period exhibited by the plants as compared with those grown in the greenhouse. This is not considered a contradiction of the findings that slow growth favors retention of systemic compounds in a plant. It indicates that, under these adverse conditions for germination and early growth, decomposition takes place from the time of planting. The Thimet, which hydrolyzes under alkaline conditions, may have undergone some breakdown for this reason. Conditions were also favorable to leaching of the Thimet by water action. A reduction in stand was clearly visible in the planting of treated seed.

FIELD TEST, FALL OF 1956.—Four tests were made with treated Yogo wheat planted between September 15 and 19. The dosage was ½ pound of Thimet per 100 pounds of seed, in the same carbon formulation as before, and the planting rate was 1 bushel (60 pounds) to the acre. In each test one drill width of treated seed was planted along ¼ or ½ mile of margin, with the farmer's equipment. Owing to delay in receipt of treated seed, planting was late and natural mortality of the grasshoppers reduced populations so that data were obtained from only one test, near Conrad, Montana. At this location the grasshopper population in adjacent stubble was estimated to be 5 per square yard and the species was dominantly the migratory grasshopper.

Table 2.—Effect of temperature on plant growth and on mortality of adult two-striped grasshoppers when allowed to feed on wheat grown from Thimet-treated seed.

Plants		Thimet per 100 Pounds of Seed (lb.)	Number of		Per Cent Mortality After (days)			
Age (Days)	Height (Inches)		Tests	Insects	3	4	5	10
Plants Grown at 70°–78° F. (Planted March 5)								
22	6–8	0.5	4	8	75	100	—	—
		.25	4	8	25	63	63	100
27	7–9	.5	4	16	94	94	100	—
		.25	2	8	63	63	63	100
Plants Grown at 70°–90° F. (Planted March 28)								
22	10–13	0.5	1	6	50	50	50	83
	11–15	.25	1	6	17	33	33	50
28	9–13	.5	2	12	8	25	25	58
	6–13	.25	2	12	0	8	8	8

The seedlings emerged in 5 to 7 days, and two to three drill rows were eaten to ground level in the first 3 days on both treated and untreated strips. After 14 days the damaged plants on both plots showed recovery and had added 1 inch of growth. Undamaged plants were 3 to 4 inches high at this time. Dying and freshly killed grasshoppers were present on the treated plot, and it was estimated that there was only one live grasshopper left to each 4 square yards of margin, whereas there were from 1 to 5 per square yard feeding on the seedlings in the untreated plot. After 27 days good recovery had taken place on the treated plots, and plants were in the three-leaf stage. At this time all plants in the first two to four rows of untreated margin had been eaten to ground level but did not appear dead.

In this test most of the damaged seedlings in the untreated check strip recovered the following spring. A nearby field that had been planted about 2 weeks earlier showed no recovery, and 10 to 20 feet of all margins had been destroyed. Owing to dry fall weather some seed along the margins failed to germinate until spring. This and the recovery of damaged plants obscured the results obtained in the fall; however, dead plant stubs could be found along the untreated margin but none along the treated side. No kill was obtained in the spring, when treated plants were fed to adult two-striped grasshoppers.

LABORATORY TESTS, 1957.—The principal purpose of the tests in 1957 was to determine the number of systemically treated wheat seedlings that could survive heavy grasshopper feeding during the early stage of growth. An initial large grasshopper population feeding on an average stand of winter wheat seedlings was simulated.

Seed treatments with Thimet at ½ pound and Bayer 19639 at 1 pound per 100 pounds of seed were compared with granule applications of these compounds at ½ and

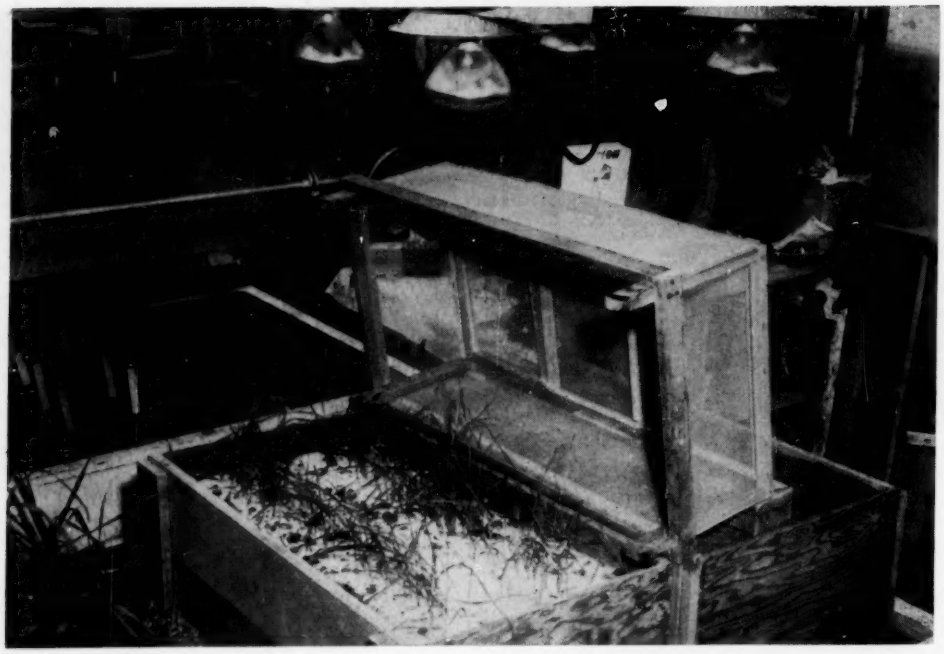

FIG. 2.—Greenhouse flat used in 1957, showing removable screen cage and overhead heat-lamp arrangement.

1 pound per acre, respectively. Thimet was applied to the seed as an activated-carbon formulation with a methyl cellulose sticker. Bayer 19639 was applied as a drench, and the seed was coated with highly absorbent kaolin to absorb the excess toxicant. The granule formulation of Thimet contained 2% of toxicant, and that of Bayer 19639 2.5%, and both were applied at seed depth.

For each material and formulation 125 seeds of Yogo winter wheat were planted in two rows in flats 36 by 18 inches and 15 inches deep. This number of seeds was calculated to produce a stand comparable to field conditions. A removable screen cage was placed over each flat, and adult two-striped grasshoppers were introduced daily the first week and at intervals thereafter. Two 250-watt reflector-type infrared lamps, controlled by a time clock, furnished heat and light to provide good feeding conditions for 8 hours of each day (fig. 2). Results are shown in table 3.

Both materials and formulations gave 100% mortality of introduced grasshoppers in 3 days through the fourth week, but during the fifth week 10 days were required to obtain the indicated mortalities. The number of plants was materially reduced by both seed treatments but not by the granule application. At the dosages used plant survival from treatment with Thimet and Bayer 19639 was equally good.

Previous tests indicated that germination of treated wheat seed is a major problem. Treated and untreated seeds from one lot of Yogo retained from those tests were germinated between moistened blotter paper in an incubation chamber and the results compared with the number of plants emerging from soil.[2] Two hundred seeds were used in each blotter test and 125 in each soil test. Seed treatments with each compound were the same as described previously. Germination from soil was based on the number of plants that emerged. These tests were preliminary in nature and only limited conclusions are justified. The results are presented in table 4.

No material reduction in germination resulted from either Thimet or Bayer 19639 when applied as granules. When used as seed treatments, both compounds reduced germination materially when results were based on emergence from soil but only moderately so when tested by the standard laboratory method. The standard laboratory test was obviously a poor index of the stand to be expected from treated seed.

DISCUSSION.—In the laboratory tests treated plants retained good toxicity to grasshoppers for 4 weeks and reached the three-leaf stage with little damage. The number of insects used in each test was believed to exceed that normally found in the field during the fall growing season, and use of this method appears practical. Under fall field conditions rate of growth is slower than under the conditions of the laboratory tests; consequently degradation of the insecticide is reduced and toxicity maintained at a high level. Extended delay in emergence, such as results from cool, wet spring weather, materially reduced the period during which the plants were toxic to grasshoppers.

Wheat seed appears to be less tolerant of these systemic compounds than other seed, such as cotton, and stands will be materially reduced by some type of seed treatment. This problem can probably be solved by use of granules or mixtures of the insecticide in fertilizer applied in the furrows, but equipment is often unavailable or there may be no need for use of fertilizer. A better

[2] Tests conducted by the Montana State College Grain Laboratory according to the rules for testing seeds adopted by the Association of Official Seed Analysts (July 1, 1955).

Table 3.—Protection afforded winter wheat seedlings by seed and granule treatments with Thimet and Bayer 19639.

Treatment	Grasshoppers: Number Added in 1st Week	Grasshoppers: Number Added in 4 Weeks	Grasshoppers: Per Cent Mortality at 4 Weeks	Grasshoppers: Per Cent Mortality at 5 Weeks	Plants: Total Number	Plants: Per Cent Survival After 4 Weeks
Thimet: Seed[a]	18	30	100	91	47	79
	12	24	100	50	59	93
Granules	18	40	100	33	104	89
Check	18	22	—	—	110	0
Bayer 19639: Seed[a]	18	30	100	90	58	81
	18	30	100	80	72	69
Granules	18	40	100	100	108	79
Check	6	6	—	—	100	0

[a] Two tests were conducted with seeds but only one with granules.

Table 4.—Germination of wheat after seed and granule applications of Thimet and Bayer 19639.

Treatment Pounds per 100 Pounds (seed) or per Acre (granules)	Days in Storage After Treatment	Per Cent Germination: Treated	Check	Reduction
		Emergence from soil		
Thimet:				
Granules 0.5	203	83	—	—
Seed coat 0.5	159	38	88	50
	175	47	80	33
	231	40	74	34
		Laboratory test		
0.5	149	92	91	0
	213	89	97	8
		Emergence from soil		
Bayer 19639:				
Granules 1	39	86	—	—
Seed coat				
1	48	60	74	14
2	60	69	89[a]	20
4	60	54	89[a]	35
		Laboratory test		
1	30	82	97[b]	15
2	30	79	97[b]	18
4	30	90	97[b]	7

[a] One check against both treatments.
[b] One check against all treatments.

approach will be the development of a seed treatment that will not be toxic to the seed.

The field tests indicate that the amount of damage done by initial feeding would not interrupt continuous growth. Under favorable conditions winter wheat in Montana will emerge in 5 to 7 days and reach the three-leaf stage in 2 to 3 weeks after seeding. The toxic period therefore appears to be adequate for the plants to become established.

Fall grasshopper populations along wheat margins are governed by the numbers and the food plants in adjacent habitats or by flights from other areas. All this pressure is not exerted at the same time, and since the plants can withstand some early feeding, the respite given by treated plants allows them to reach a stage of growth where feeding by grasshoppers no longer has any material effect.

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Residual Behavior of Various Insecticides on and in Lemons and Oranges[1]

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Abstract

Magnitudes and persistence of chlordane, dieldrin, and heptachlor residues on and in lemons, and of malathion residues on and in Valencia oranges, are recorded. Persistence half-life values of these insecticides in the peel of the fruit are 19, 60, 23, and 32 days, respectively. Only negligible apparent residues were found in the edible portion of the fruit.

In order to record data accrued for the purpose of supporting petitions for tolerances under Public Law 518, (Miller Amendment) residue data and half-life values (Gunther & Blinn 1955) for chlordane, dieldrin, and heptachlor on and in lemons, and for malathion on and in Valencia oranges, are presented.

Materials and Methods.—Applications of each material were made as full-coverage sprays, by means of a high-pressure reciprocating pump and manually operated spray guns. Mature fruits were picked, one from each quadrant of each of eight trees in each plot, and the resulting 32 fruits were processed as a unit. Replicate 32-fruit samples for each sampling date were processed separately.

All the fruits from each replicate were peeled, and 1-pound subsamples of the minced peel and of the minced pulp were processed separately with *n*-hexane for the chlordane-, dieldrin-, and heptachlor-treated fruit, and with redistilled carbon tetrachloride for the malathion-treated fruit, in the manner and with the equipment previously described by Gunther & Blinn (1955) to afford final stripping solutions. Aliquots of the stripping solutions were assayed by the combustion total-chloride method for chlordane, dieldrin, and heptachlor (Gunther & Blinn 1955), after being washed free of inorganic chloride, and by the colorimetric procedure for malathion (Norris *et al.* 1954).

Results.—Magnitudes of chlordane residues on and in peel of field-treated lemons are presented in table 1. Residue values for apparent chlordane in the pulp (edible portion) of the fruit over the 34-day sampling period never exceeded 0.1 p.p.m. after corrections for pulp background and recovery.

Magnitudes of dieldrin residues on and in peel of field-treated lemons are presented in table 2. Residue values for apparent dieldrin in the pulp (edible portion) of the fruit over the 34-day sampling period never exceeded 0.1 p.p.m. after corrections for pulp background and recovery.

Table 1.—Residues of chlordane on and in peel of field-treated lemons.[a]

Days After Application[b]	P.P.M. Chlordane[c]	
	Average	Range
7	15.0	12.5–16.4
14	12.0	9.1–14.4
21	9.0	6.9–10.6
27	7.2	5.4– 9.5
34	5.6	5.2– 6.2

[a] Based on weight of peel only. Mature lemons have 30.0± 8.5 wt. per cent peel as determined by 632 measurements.
[b] Treated with 4 pounds of wettable powder (50% chlordane) per 100 gallons of water on August 18, 1955.
[c] Values are for three replicates and are corrected for background (0.24 p.p.m.) and for recovery (90%).

Table 2.—Residues of dieldrin on and in peel of field-treated lemons.[a]

Days After Application[b]	P.P.M. Dieldrin[c]	
	Average	Range
7	31.4	28.0–36.8
14	13.7	10.5–16.1
21	7.3	5.7– 8.7
27	8.7	7.8– 9.4
34	6.2	5.9– 6.1

[a] Based on weight of peel only. Mature lemons have 30.0± 8.5 wt. per cent peel as determined by 632 measurements.
[b] Treated with 2 pounds of wettable powder (50% dieldrin) per 100 gallons of water on August 18, 1955.
[c] Values are for three replicates and are corrected for background (3.0 p.p.m.) and for recovery (90%).

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Table 3.—Residues of heptachlor on and in peel of field-treated lemons.[a]

Days After Application	P.P.M. Heptachlor[b]			
	4 Lbs. W.P.[c]		2 Lbs. W.P.[d]	1 Qt. E.C.[e]
	Average	Range		
0[f]	—		3.8	5.4
2	—		2.1	2.2
5	—		2.7	1.1
7	8.4	6.8–10.2	—	—
9	—		3.2	1.5
14	3.2	2.7– 3.6	—	—
15	—		2.5	1.0
21	3.6	3.3– 4.3	—	—
27	2.9	2.7– 3.2	—	—
30	—		1.6	0.6
34	1.7	1.5– 1.9	—	—
60	—		0.8	0.3

[a] Based on weight of peel only. Mature lemons have 30.0± 8.5 wt. per cent peel as determined by 632 measurements.
[b] All values are corrected for background (0.14 p.p.m.) and for recovery (90%).
[c] Values are for three replicates of fruit treated with 4 pounds of wettable powder (25% heptachlor) per 100 gallons of water on August 18, 1955.
[d] Each value represents a single sample of fruit treated with 2 pounds of wettable powder (25% heptachlor) per 100 gallons of water on October 27, 1954.
[e] Each value represents a single sample of fruit treated with 1 quart of emulsifiable concentrate formulation (2 pounds heptachlor per gallon) per 100 gallons of water on October 27, 1954.
[f] Sampled as soon as spray residues had dried.

Table 4.—Residues of malathion on and in peel of field-treated Valencia oranges.[a]

Days After Application[b]	P.P.M. Malathion[c]	
	Average	Range
1	3.5	3.1–4.1
2	2.9	2.4–3.2
7	1.5	1.4–1.6
14	1.0	1.0–1.1
21	0.5	0.5–0.6
28	0.4	0.3–0.6
62	0.2	0.1–0.2

[a] Based on weight of peel only. Mature Valencia oranges have 18.7 ± 6.3 wt. per cent peel as determined by 297 measurements.
[b] Treated with 3 pounds of wettable powder (25% malathion) per 100 gallons of water on August 31, 1954.
[c] Values are for three replicates and are corrected for background (0.1 p.p.m.) and recovery (88%).

Table 5.—Half-life values, in days, for chlordane, dieldrin, and heptachlor on and in peel of field-treated lemons, and for malathion on and in peel of field-treated Valencia oranges.

Insecticide	Formulation	Half-Life Values, Days	
		Degradation	Persistence
Chlordane[a]	4 lb. W.P.	—	19
Dieldrin[b]	2 lb. W.P.	6.5	60
Heptachlor[c]	4 lb. W.P.	—	15
	2 lb. W.P.	—	27
	1 qt. E.C.	3.5	25
Malathion[d]	3 lb. W.P.	7	32

[a] See table 1 for exact formulation.
[b] See table 2 for exact formulation.
[c] See table 3 for exact formulation.
[d] See table 4 for exact formulation.

Magnitudes of heptachlor residues on and in peel of field-treated lemons are presented in table 3. Residue values for apparent heptachlor in the pulp (edible portion) of the fruit over the 60-day sampling period never exceeded 0.2 p.p.m. after corrections for pulp background and recovery.

Magnitudes of malathion residues on and in peel of field-treated Valencia oranges are presented in table 4. Residue values for apparent malathion in the pulp (edible portion) of the fruit over the 62-day sampling period never exceeded 0.04 p.p.m. after corrections for pulp background and recovery.

DISCUSSION.—By plotting parts-per-million values *vs.* elapsed days since treatment on linear coordinates, regular exponential-type curves are obtained, whereas logarithmic plotting of the same residue values affords straight lines (Gunther & Blinn 1955). Both graphical representations of residual behavior are illustrated in figure 1 by the data for malathion residues on and in peel of field-treated Valencia oranges. The two intersecting straight lines resulting from the plotting of these residue data using a logarithmic coordinate represent the attenuation of the initial deposit and of the true residue, respectively. Thus the initial rapid dissipation (degradation) of the chemical occurs while it remains principally on the surface and is subject to physical and chemical diminution by weathering, whereas the later, less rapid dissipation (persistence) results from penetration of the remaining chemical into the waxes and oils of the fruit, where its decrease in concentration is due primarily to metabolic attack and increase in fruit size. Each of these lines or types of residue behavior can be characterized by a half-life value, *i.e.*, the time required for half of the residue to be dissipated.

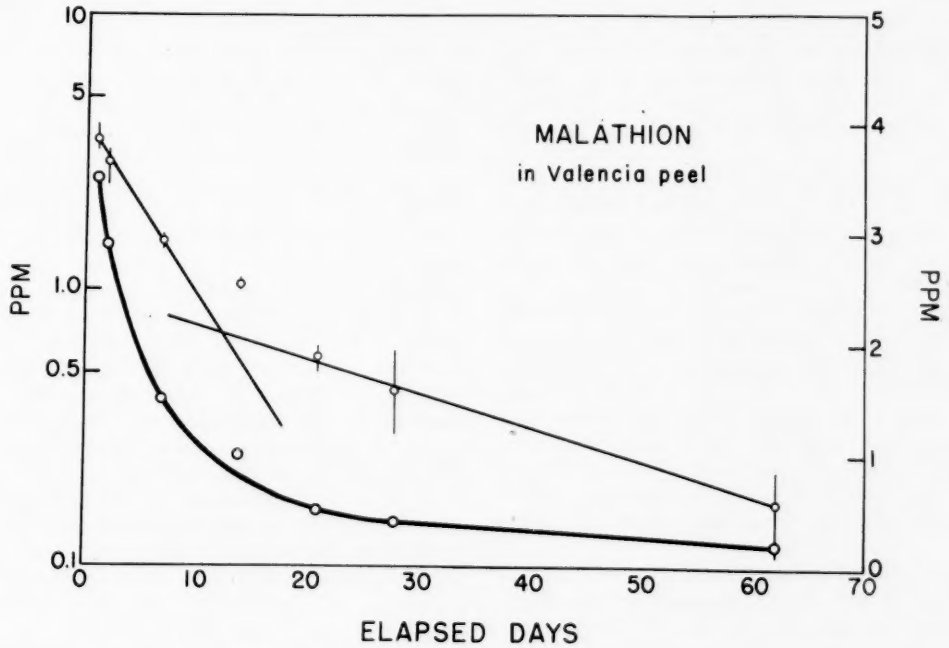

FIG. 1.—Residual behavior of malathion on and in peel of field-treated Valencia oranges (○——○ logarithmic coordinate; ○——○ linear coordinate).

Half-life values for the insecticides reported here are listed in table 5. The residual behavior in terms of half-life values is in agreement with that established for other insecticides (Gunther & Blinn 1955, 1956).

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Toxic Action of Dipterex and DDVP to the House Fly[1]

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ABSTRACT

Dipterex or *O,O*-dimethyl 1-hydroxy-2,2,2-trichloroethyl phosphonate under mildly alkaline conditions is rapidly degraded to DDVP. The half-life values for this reaction are: pH 8.0—63 minutes, pH 7—386 minutes, and pH 6.0—89 hours. The rate of reaction is very slow at pH 5.4. The *in vitro* inhibition of house fly head cholinesterase by 10^{-7} M Dipterex showed marked pH dependency and ranged from 11% at pH 5.4 to 100% at pH 8.0, thus clearly demonstrating that DDVP formation is necessary for *in vitro* cholinesterase inhibition.

In vivo studies of the mode of action of Dipterex showed that the rate of knockdown of house flies feeding on Dipterex-treated moist sugar bait was much more rapid at pH 7.0 than pH 5.4. This, together with the 4- to 7-fold greater toxicity of DDVP over Dipterex and the isolation of about 5% of P^{32} DDVP from the total P^{32} metabolites in Dipterex-poisoned house flies, strongly indicates that DDVP is responsible for the *in vivo* toxic action of Dipterex.

Dipterex[2] or *O,O*-dimethyl 1-hydroxy-2,2,2-trichloroethyl phosphonate (Bayer L 13/59) has proved of spectacular value as a toxicant in baits and bait sprays for *Musca domestica* L., and other flies. The experiments in this paper were directed to the elucidation of the mode of action of this compound. The compound DDVP or dimethyl 2,2-dichlorovinyl phosphate has been shown to exist in Dipterex as an impurity and to be produced by rearrangement under alkaline conditions (Lorenz *et al.* 1955; Barthel *et al* 1955; and Mattson *et al.* 1955). DDVP is extremely toxic to flies and it seemed logical that this compound might be involved in the mode of action of Dipterex.

MATERIALS AND METHODS.—Dipterex was obtained from the Chemagro Corporation and was recrystallized three times from petroleum ether to give a white crystalline powder m.p. 75–6° C. DDVP was prepared from this by the sodium hydroxide treatment of Mattson *et al.* (1955) and distilled to give a product b.p. 65–6° C. (0.1 mm.) $n_D^{27.5°}$ 1.4513. A sample of P^{32}-labeled Dipterex, 99+% pure by paper chromatography was kindly furnished by Dr. Gerhard Schrader of Farbenfabriken Bayer. This product had a relative activity of 11.3 c.p.s. per γ, and a portion was converted to P^{32}-labeled DDVP by the method previously mentioned, with a relative activity of 13.1 c.p.s. per γ. The preparation of P^{32}-labeled Dipterex and DDVP has been described by Acree *et al.* (1956).

The topical and oral applications to flies were made by a micrometer-driven 0.25 ml. tuberculin syringe, applying 1λ of acetone solution to each female fly anesthetized with carbon dioxide. Paper chromatography was carried out using Whatman No. 1 paper impregnated with 50% propylene glycol and mobile solvents of (a) Skellysolve B 4 parts and toluene 1 part saturated with propylene glycol or (b) benzene saturated with propylene glycol. Cholinesterase activity was measured manometrically as described by Metcalf & March (1950) or by the colorimetric method of Hestrin (1949) as described by Metcalf (1951).

Radioassay of the P^{32} Dipterex and DDVP both in the insects and on the paper chromatograms was carried out by Geiger counter as previously described (March *et al.* 1954). Wet-ashing of treated flies with HNO_3 was attempted, but controlled experiments showed that approximately 82% of the total P^{32} was lost by this process. Therefore, the radioactivity in treated flies was measured by direct counting in planchets. Self-absorption of the strong beta-emission of the P^{32} was evaluated as subsequently described and was not an important source of error.

Hydrolysis rates of DDVP at varying hydrogen ion concentrations were carried out using P^{32}-labeled compound at 37.5° C. DDVP was shown to partition in favor of chloroform over water in the ratio of greater than 100 to 1. The labeled part of the hydrolysis products, dimethyl phosphoric acid, was found to partition in favor of water over chloroform in the ratio of greater than 100 to 1. Therefore, it is possible to determine the degree of hydrolysis by partitioning the reaction mixture between the two solvents and measuring the radioactivity in each solvent. Dilute solutions of DDVP were made up in Sorenson's phosphate buffer. It was not necessary to know the

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[2] Trademark for Chemagro Corporation, N. Y.

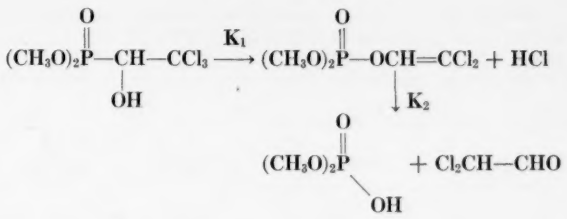
exact concentration since the reaction proceeded at a pseudo first-order rate. At measured intervals, small samples were removed, added directly to equal volumes of chloroform and water in a separatory funnel, shaken vigorously and the two phases separated. Appropriate aliquots were taken from each phase and the radioactivity determined by a thin-window Geiger tube. The data gave good first-order plots and the hydrolysis constant, K_1, was calculated from the slope of the straight line obtained.

The rate of conversion of Dipterex to DDVP was estimated by three different methods as follows. In the first method paper chromatography was employed using Whatman No. 1 paper impregnated with 50% propylene glycol in alcohol and a solvent of benzene saturated with propylene glycol. The tracer materials were shown to be 99+% pure by paper chromatography and the average Rf values were: DDVP 0.89, Dipterex 0.16, and dimethyl phosphoric acid, the hydrolysis product of DDVP, 0.0. Other systems for the paper chromatography of these compounds have been described by Robbins *et al.* (1956). The rates of transformation of Dipterex in Sorenson's phosphate buffer (solutions) were followed at 37.5° C. by serial counting of the P^{32} spots as described by Fukuto & Metcalf (1954). By this method both decomposition of Dipterex to DDVP and DDVP to dimethyl phosphoric acid could be determined. P^{32}-labeled material was also used in the second method in which Dipterex and DDVP were differentiated by solvent partition between carbon tetrachloride and water. DDVP was found to partition in favor of carbon tetrachloride to water in the ratio of 25 to 1 and Dipterex in favor of water to carbon tetrachloride in the ratio of 12 to 1. Since appreciable amounts of Dipterex partitioned into carbon tetrachloride, it was necessary to make adjustments in the amounts found. This method could only be used to estimate the relative amount of DDVP and the initial decomposition rate of Dipterex since dimethyl phosphoric acid eventually ended up in the aqueous phase. In the third method the conversion rate of Dipterex was followed by chloride ion estimation. Solutions of 0.01 *M* Dipterex in Sorenson's phosphate buffer were made up and at measured time intervals aliquots were taken and the chloride ion estimated by the Volhard method.

The rate of reaction of Dipterex and its *N*-phenyl carbamate with triethylamine in absolute methyl alcohol was also determined by the Volhard technique. The solutions were made up 0.01 *M* in compound and 0.02 *M* in triethylamine.

1-Dimethoxyphosphinyl-2,2,2-trichloroethyl-1 N-phenylcarbamate (*Dipterex carbamate*).—A mixture of 1 gm. Dipterex, 0.5 ml. phenyl isocyanate, and two drops boron trifluoride etherate was heated on a steam bath for 2 hours. The mixture when cooled gave crystals which after two recrystallizations from ether and n-hexane melted at 155–158°. Elemental analysis: per cent calculated for $C_{11}H_{13}Cl_3NO_5P$, C=35.08; H=3.48; found, C=34.69, H=3.85.

EXPERIMENTAL.—*The conversion of Dipterex to DDVP.*—The conversion of Dipterex to DDVP and the subsequent hydrolysis of DDVP according to the equations shown below were investigated at pH 5.4, 6.0, 7.0 and 8.0 in Sorenson's phosphate buffer.



There is a large difference in the rate of conversion of Dipterex even under these small pH changes. The curves in figure 1 show the relative rates of decomposition as measured by chloride ion determination. At pH 8.0 decomposition occurred quite rapidly with 60% reaction after 2 hours. In comparison at pH 7.0 about 11 hours was required to obtain 60% decomposition. At pH 6.0 the reaction was very slow and was practically nil at 5.4. Kinetic analysis of the data obtained showed that the reaction proceeded as a pseudo first-order reaction up to about 20 to 25% reaction and then slowed down at a gradual rate, possibly because the buffering capacity of the buffer was exceeded. The initial first-order rate constants (K_1) for the dehydrochlorination of Dipterex and presumably the formation of DDVP are given in table 1. At pH 6.0, however, the rate of chloride ion formation dropped off rapidly after about 10% reaction and the first-order rate constant is not a good measure of the velocity of the reaction here.

The rates of hydrolysis of DDVP in the same buffers were determined using P^{32}-labeled material. The rate runs were carried out in extremely dilute solutions of DDVP in buffer and the data showed that the hydrolysis proceeded according to the first-order law. The calculated first-order hydrolysis constants, K_2, are given in table 1. Mühlmann & Schrader (1957) have also determined rate constants for the hydrolysis of Dipterex and DDVP at various pH values. Their results which were obtained by

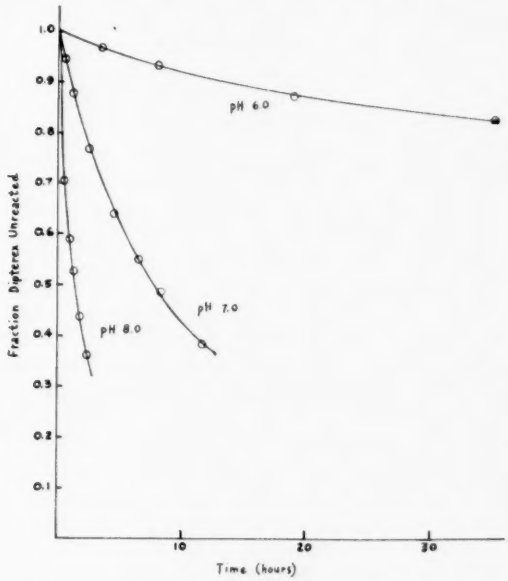


FIG. 1.—Decomposition of Dipterex at differing pH's as determined by chloride ion liberation.

Table 1.—Rate constants for the dehydrochlorination of Dipterex (K_1) and for the hydrolysis of DDVP (K_2) in phosphate buffers of varying pH and at 37.5° C.

	Dipterex		DDVP	
pH	K_1 (Min.$^{-1}$)	t ½ Min.	K_2 (Min.$^{-1}$)	t ½ Min.
8.0	1.1×10^{-2}	63	2.3×10^{-3}	301
7.0	1.8×10^{-3}	386	1.5×10^{-3}	462
6.0	1.3×10^{-4}	5340 (89 hr.)	3.3×10^{-4}	2100 (35 hr.)
5.4	—		1.5×10^{-4}	4620 (77 hr.)

a different method are approximately ten-fold greater than the results presented here, which were confirmed by the close agreement of three independent methods.

By using P^{32}-labeled Dipterex it was possible to determine the relative amount of DDVP present in the reaction mixture. Curves showing the rates of decomposition of Dipterex and formation of DDVP at pH 8.0 are given in figure 2. It can be seen that initially the amount of DDVP builds up rapidly at a rate equal to the disappearance of Dipterex. The concentration of DDVP reaches a maximum at about 2.5 hours (55%) at which time only about 30% of the original Dipterex is left. The amount of DDVP then diminishes gradually as it is hydrolyzed to dimethyl phosphoric acid. Also shown in figure 2 is the curve showing the relative amounts of DDVP calculated from the two rate constants K_1 and K_2. Since K_1 deviated from the first-order law after 25% reaction the calculated values would be expected to be greater than the experimental. In addition, the curve showing the formation of dimethyl phosphoric acid as calculated from K_1 and K_2 is given.

Figure 3 shows similar curves depicting the conversion of Dipterex and formation of DDVP at pH 7.0. At pH 7.0 the maximum concentration of DDVP was obtained after 12 hours.

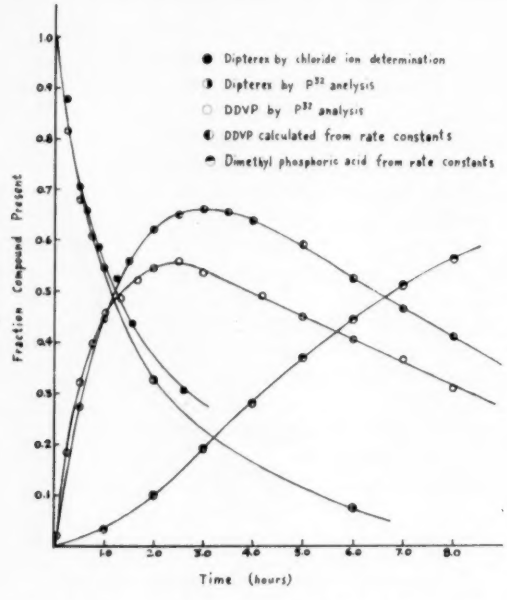

Dipterex by chloride ion determination
Dipterex by P32 analysis
DDVP by P32 analysis
DDVP calculated from rate constants
Dimethyl phosphoric acid from rate constants
Fraction Compound Present
Time (hours)

FIG. 2.—Decomposition of Dipterex and formation of DDVP at pH 8.0.

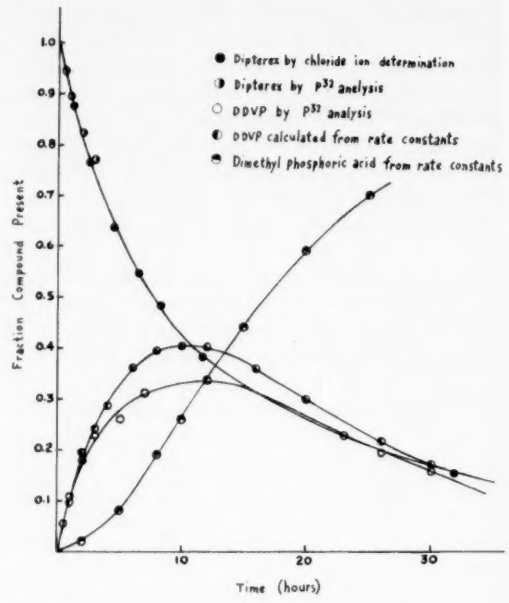

Dipterex by chloride ion determination
Dipterex by P32 analysis
DDVP by P32 analysis
DDVP calculated from rate constants
Dimethyl phosphoric acid from rate constants
Fraction Compound Present
Time (hours)

FIG. 3.—Decomposition of Dipterex and formation of DDVP at pH 7.0.

The rearrangement of Dipterex to DDVP via dehydrochlorination is an unusual reaction and some effort was made to elucidate the mechanism of this rearrangement. It was found that Dipterex reacts with organic bases in anhydrous methyl alcohol liberating chloride ion. The rate of chloride ion liberation appears to be a function of the strength of the organic basic, *e.g.*, more chloride ion is liberated in 10 minutes with triethylamine, a relatively

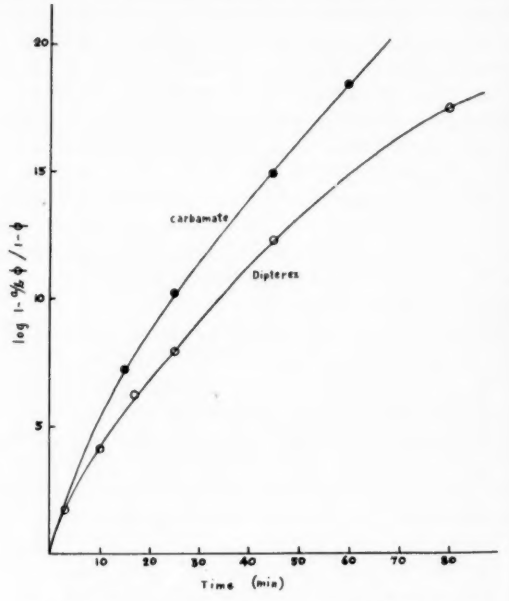
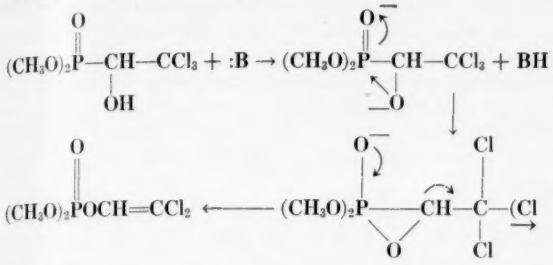
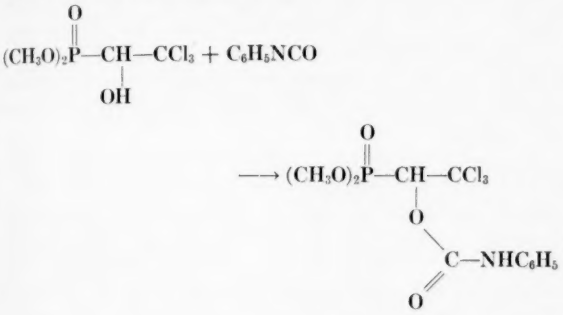


FIG. 4.—Reaction between Dipterex and Dipterex carbamate with triethylamine in methanol.

strong organic base, than occurs in 2 weeks with pyridine, a weak base. It seems, therefore, that the reaction is dependent on the rate of removal of a proton. One might expect that the reaction proceeds as follows:



It was felt that the mechanism suggested would be strengthened if it could be demonstrated that a compound in which the hydroxyl group in Dipterex is blocked showed little or no reaction with triethylamine. Dipterex was treated with phenyl isocyanate to give the corresponding *N*-phenylcarbamate.



However, this compound reacted faster with triethylamine than Dipterex under comparable conditions. Second-order plots are shown in figure 4 where ϕ is the fraction of compound reacted, "a" the initial concentration of compound, and "b" the initial concentration of triethylamine. This indicates that with the carbamate the γ-hydrogen atom is removed. The product from this reaction has yet to be identified and it is not clear whether the carbamate also rearranges to DDVP; but, as will be mentioned later, the compound is not appreciably toxic to house flies. Further work on the mechanism of rearrangement of Dipterex to DDVP is in progress.

ANTICHOLINESTERASE ACTIVITY OF DIPTEREX.—From the general picture of the mode of action of the organophosphorus compounds (Metcalf 1955, Fukuto 1957) it seems highly probable that intoxication by DDVP and Dipterex is the result of progressive inhibition of insect cholinesterase. This was evaluated by exposing flies on the Dipterex impregnated disc for 40 to 50 seconds until they were paralyzed, immediately removing their heads, and homogenizing in 0.025 M $NaHCO_3$ buffer. This brei was immediately evaluated for cholinesterase inhibition by the standard manometric technique (Metcalf & March 1950) and it was found that the enzyme was almost completely (99+%) inactivated within the period of about 30 minutes required for the entire operation from poisoning to measurement.

The *in vitro* anticholinesterase actions of Dipterex and DDVP were compared using purified samples of each. With the standard manometric procedure using a brei of three house-fly heads per ml. and acetyl choline bromide at a final concentration of 0.01 M it was found that over a 45-minute period Dipterex produced 50% cholinesterase inhibition at 1.5×10^{-8} M and the DDVP at 8×10^{-9} M. Thus DDVP is apparently about five times as effective as Dipterex as a cholinesterase inhibitor. The values found for the inhibition of fly head cholinesterase indicated considerably higher activity than that demonstrated by Arthur & Casida (1957) to purified acetylcholine esterase.

Table 2.—Inhibition of fly brain cholinesterase by Dipterex and DDVP at various pH values.

pH	μ M ACh. Hydrolyzed/Ml. by Control	Per Cent Cholinesterase Inhibition: Dipterex 1 $\times10^{-7}$ M	Per Cent Cholinesterase Inhibition: DDVP 1 $\times10^{-8}$ M
5.4	0.18	11%	100%
6.0	0.70	46	100
7.0	1.12	86	100
8.0	1.06	100	100
9.4	0.99	100	100

Although Dipterex has been suggested as acting as a direct cholinesterase inhibitor (Arthur & Casida 1957), a consideration of its phosphonate structure characterized by the P—C bond does not suggest any mechanism by which the compound may be cleaved by reaction with the cholinesterase enzyme as is essential for irreversible phosphorylation (Fukuto & Metcalf 1956). This consideration does not apply to DDVP in which the relatively labile P—O—C—bond replaces the phosphonate (P—C) bond of Dipterex.

It has been shown that Dipterex rearranges rapidly to DDVP under mildly alkaline conditions and it seemed possible that this rearrangement might account for both the *in vivo* toxicity of Dipterex and for its apparent *in vitro* anticholinesterase activity. This point was investigated using the Hestrin colorimetric technique to measure the inhibition of fly brain cholinesterase at varying pH values. Sorenson's phosphate buffers were employed at pH 5.4, 6.0, 7.0, 8.0, and 9.4 and Dipterex at 1×10^{-7} M and DDVP at 1×10^{-8} M were used as inhibitors. The results obtained are shown in table 2. The experiment is complicated because of the marked variation in cholinesterase activity with pH, the optimum being between 7 and 8; but shows conclusively that DDVP inhibits strongly at either acid or alkaline pH, while Dipterex is only feebly inhibitory at pH 5.4, but becomes much more active at higher pH values. This information, together with the fact that Dipterex has been shown above to be fairly rapidly converted to DDVP at both pH 7 and pH 8, indicates that the *in vitro* anticholinesterase activity shown by purified Dipterex at the pH range of 7.4–7.6 as commonly employed in manometric or electrometric assay is a result of the formation of DDVP. This is in accord with the results recently found by van Die (1957), and is supported by the data of Arthur & Casida (1957) who found that the rate of recovery of rat erythrocyte cholinesterase inhibited with either Dipterex or

DDVP was almost identical, indicating that the same dimethyl phosphoryl cholinesterase was formed with each compound. This information has an important bearing on the interpretation of residue analyses for Dipterex by the cholinesterase method.

That DDVP is the active intermediate in Dipterex intoxication was further established by experiments in which the *in vivo* formation of DDVP in house flies feeding on the P^{32} Dipterex was demonstrated. Several hundred starved house flies (susceptible strain) were allowed to feed on a sugar solution containing 2% P^{32} Dipterex. The solution was made up in pH 5.4 phosphate buffer to minimize base catalyzed formation of DDVP. After complete knockdown (ca. 1 hour), the flies were thoroughly washed with acetone and ground with anhydrous sodium sulfate under *n*-hexane in a mortar. The *n*-hexane extract was concentrated under vacuum, the oily residue taken up in a few drops of acetonitrile and applied to propylene glycol treated paper. Development of the paper chromatograms with the usual solvent showed that about 5% of the P^{32}-labeled material isolated by this procedure cochromatographed with DDVP (R_f 0.89). The radioactive section on the region of R_f 0.89 was sectioned, eluted with acetone and fly-brain anticholinesterase measurements made. This substance completely inhibited cholinesterase at 10^{-7} M, which further supports its identity as DDVP.

RELATIVE TOXICITY OF DIPTEREX AND DDVP.—The relative contact and oral toxicities of Dipterex were determined by the topical application of 1 microliter droplets of standard w/v acetone solutions to the thoraces of groups of 20 female, 2 to 4 days old, *Musca domestica* and by the application of equal volumes of w/v aqueous solutions in 10% sugar water directly upon the labella of partially anesthetized flies which were then observed under a binocular microscope until complete ingestion occurred. The values obtained were: Dipterex—oral LD_{50} between 0.01–0.03γ per fly, topical LD_{50} 0.2γ per fly; DDVP topical LD_{50} 0.028γ per fly. Thus Dipterex was about 10 times as effective orally as topically, and DDVP was approximately seven times as toxic topically as was Dipterex. The topical LD_{50} values agreed very well with the respective values of 0.2 and 0.022γ given by Mattson *et al.* (1955). Arthur & Casida (1957) found comparable toxicities of about 0.22γ per fly (11γ per g.) for Dipterex, and 0.012γ/fly (0.6γ/g.) for DDVP.

The greater effectiveness of Dipterex after oral administration is possibly due to its predominantly polar nature (partition coefficient for carbon tetrachloride/water—1:14 as compared with 28:1 for DDVP) which results in a low rate of cuticular absorption, rather than from an accelerated rate of conversion in the digestive tract. The pH of the house fly foregut and midgut, as determined by feeding phenol red and bromothymol blue, is slightly acidic, from about 6.5 to 7.0. Therefore, it is unlikely that the rate of conversion of Dipterex to DDVP in the gut is appreciably more rapid than in the general body tissues.

In order to approach this subject in another way, the radioactive tracer preparations were used. Small crystals of P^{32} Dipterex were dissolved in 40% sugar water on filter paper and while still moist were exposed to female flies starved 2 days and confined under a petri dish cover. The flies were removed as soon as they were paralyzed and the amount of radioactivity calculated as Dipterex was measured by direct counting with a Geiger counter. A series of 10 flies individually measured, accumulated from 0.12 to 0.41γ per fly with an average LD_{100} of 0.25γ per fly which demonstrates the efficiency of the bait method. The same 10 flies were homogenized in water and 1 ml. aliquots counted after evaporation in the planchet. By this technique the average value per fly was 0.29γ Dipterex indicating that self absorption of the P^{32} is not a source of large error in determining the activity per fly.

The vapor toxicity of P^{32} DDVP was evaluated by placing about 1 mg. on a 9-cm. filter paper in a petri dish and confining flies in a screened cage directly above, but not in contact with the paper. The flies were counted for radioactivity as soon as they became paralyzed. The KD_{50} was in 10 minutes and the KD_{100} in 15 minutes. The average value of DDVP accumulated for 10 flies was 0.057γ per fly by direct counting and 0.075 per fly after homogenization and evaporation of an aliquot. These LD_{100} values are in good agreement with the LD_{50} value of 0.028γ obtained by topical application and indicate that the flies tend to absorb more than a lethal dosage of the DDVP vapor before knockdown occurs. Based on the LD_{100} values, DDVP is about four times as toxic as Dipterex.

It is of interest that the *N*-phenyl carbamate of Dipterex, 1-dimethoxy-phosphinyl-2,2,2-trichloroethyl-1-*N*-phenylcarbamate was of very low toxicity when applied topically to the house fly, the LD_{50} being >500γ/g. This is in contrast to the acetyl-derivative *O,O*-dimethyl 2,2,2-trichloro-1-acetoxyethyl phosphonate which Arthur & Casida (1957) found to be approximately as toxic as Dipterex to flies topically. This suggests that the acetate which should be much more readily hydrolyzed *in vivo* than the carbamate, is readily converted to DDVP after hydrolysis.

LETHAL ACTION OF DIPTEREX.—Experiments were carried out in which individuals of a fully susceptible strain of house flies starved for 24 hours were placed on a Dipterex-sugar impregnated disc covered with a screen wire top, and the time recorded for knockdown. Data obtained from 20 normal flies showed knockdown and paralysis in from 17 to 87 seconds with an average time of 48 seconds. In order to determine if the insecticide was active by contact or by oral ingestion, the experiment was repeated with flies from which the labella had been excised. The time for incapacitation was much slower and ranged from 1 to 21 minutes for 13 flies, with an average of 10 minutes, clearly demonstrating that the major toxic action was exerted through the labellum.

The indication that Dipterex acts by conversion to DDVP suggests that the rate of knockdown of flies by Dipterex baits should be controlled by the use of buffer solutions of various pH's as wetting agents. This was investigated by moistening 5 mg. of Dipterex and an equal amount of sugar on a 9-cm. filter paper, with 1 ml. of Sorenson's buffers pH 5.4 or pH 8.6. The rate of knockdown of two replicates of 20 non-resistant flies confined under screen wire was then determined. The pH 8.6 treatment produced a KD_{50} in 1 minute as compared with times of 15 and 20 minutes for the pH 5.4 treatment.

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Granulated Insecticides for Control of the European Corn Borer in Southwestern Ontario[1]

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ABSTRACT

In 1956, sweet corn plots treated with hand applicators three times at weekly intervals with granulated endrin at 0.5 lb. of toxicant per acre or with granulated heptachlor at 1.5 lb. for control of the European corn borer (*Pyrausta nubilalis* (Hbn.)) yielded over 95% marketable ears. Plots treated with DDT emulsion, granulated DDT, and granulated toxaphene at 1.5 lb. of toxicant per acre yielded 80%, 73%, and 62% marketable ears, respectively. Only 2% of the ears in the untreated check plots were marketable. In 1957, a commercial applicator being used, granulated heptachlor at 1 lb. of toxicant per acre, granulated endrin at 0.3 lb., and granulated Dipterex at 1.5 lb. reduced the number of larvae by over 90%; a DDT emulsion spray at 1.5 lb. and granulated DDT at 1 lb., 87% and 84%, respectively. The percentage of marketable ears for the treatments were: heptachlor, endrin, and Dipterex, over 90%; DDT, over 85%; untreated check, 14%.

Farrar (1953), although working with soil-inhabiting insects at the time, stated that good control of the corn earworm (*Heliothis zea* (Boddie)) was possible when a granulated insecticide was applied to the corn plant in the late-whorl stage. This was possible because the granules temporarily remained on the leaves of the plant and then slowly, partly as a result of wind action, rolled into the whorls and leaf axils. Cox *et al.* (1956a), in Iowa, tested granulated formulations of DDT, EPN, and heptachlor. He found that "granulated formulations of insecticides are just as effective, and perhaps more effective, than emulsion sprays for control of the European corn borer."

In Ontario, dusts and sprays of DDT and ryania have been the standard insecticides for the control of the European corn borer (*Pyrausta nubilalis* (Hbn.)) for several years (Wressell 1954). Although they reduce borer populations appreciably, both insecticides have definite limitations. Ryania is expensive and obtainable in only limited quantities. Furthermore, as a wettable powder it clogs spray nozzles. DDT, on the other hand, leaves an undesirable residue on the plant. After harvest many com-

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Table 1.—Percentages of control in sweet corn plots after treatment with insecticides, Harrow, Ontario, 1956 and 1957.

Treatment	Toxicant (Lb./A.)	Infested Plants (%)	Number of Larvae		Marketable Ears (%)
			Average per Plot	Reduction (%)	
1956 Experiments					
Endrin, 2% granulated[a]	0.5	9.5	1.8	98.0	98.0
Heptachlor, 2½% granulated[b]	1.5	7.5	5.0	96.0	96.0
Toxaphene, 10% granulated[c]	1.5	67.0	9.5	92.0	62.0
DDT, 5% granulated[d]	1.5	58.5	14.5	86.0	73.0
DDT emulsion, 25%[e]	1.5	81.5	15.0	86.0	80.0
Untreated	—	99.5	112.0	—	2.0
L.S.D. at 1% level[f]			3.53		
1957 Experiments					
Heptachlor, 2½ granulated	1.0	12.5	3.3	94.0	96.0
Endrin, 1% granulated	0.3	30.0	4.0	93.0	93.0
Dipterex, 5% granulated[g]	1.5	20.0	4.8	91.0	92.0
DDT emulsion, 25%	1.5	25.0	7.3	87.0	90.0
DDT, 5% granulated[h]	1.0	46.5	8.8	84.0	85.0
Untreated	—	96.5	54.8	—	14.0
L.S.D. at 5% level[f]			6.72		

[a] Shell Chemical Corporation, New York, N. Y.
[b] Velsicol Chemical Corporation, Chicago, Ill.
[c] Hercules Powder Company, Wilmington, Del.
[d] Chipman Chemical Company, Inc., Bound Brook, N. J.
[e] Canadian Industries Limited, Montreal, Quebec, Canada.
[f] The formula used was $\sqrt{x+\frac{1}{2}}$.
[g] Chemagro Corporation, New York, N. Y.
[h] Niagara Brand Spray Company, Ltd., Burlington, Ont.

mercial growers of early table corn feed the corn stover to livestock; but if the plants have been treated with DDT, residues might occur in meat or milk, which is contrary to public health regulations. According to Fahey *et al.* (1956), working with first-brood borers in Iowa, when DDT granules were applied on field corn "the whorl, tips and leaves retained very little residue because there was no liquid to hold the particles to the leaf surface."

In Ontario, sweet corn is attacked more severely by the corn borer than field corn. When the study reported here was undertaken in1956, no reference had appeared in the literature concerning use of granulated insecticides for controlling the borer in sweet corn. More recently, Cox *et al.* (1956b) reported that in granulated and emulsion treatments at different single and multiple-application dates, DDT was better than malathion. These writers found that a single application of DDT granules was as effective as a single application of DDT emulsion, but, when multiple applications were made, emulsion sprays reduced the borer population somewhat more appreciably than the granulated form. Working in Indiana, Gould & Wilson (1957) found that single applications of several granulated formulations gave fair control of the borer in sweet corn; granulated endrin and dieldrin were better than DDT or heptachlor, but they were not so good as two applications of DDT spray.

This is a report on a 2-year study of effectiveness of several insecticides in comparison with DDT emulsion, applications being made by hand and with commercial equipment.

METHODS AND MATERIALS.—The experimental plots were on the Canada Experimental Farm, Harrow, Ont. Marcross sweet corn was planted in 1956 and Golden Beauty was used in 1957. The seed was planted 15 inches apart in rows 120 feet long, 3 feet apart. There were 6 rows in each plot. The corn was planted on May 15 in 1956, and on May 20 in 1957.

In 1956, the plots were arranged in randomized blocks of six treatments replicated four times. The insecticides and the amounts of toxicant applied per acre are shown in table 1. The granulated insecticides were formulated with Attaclay (Attapulgus Clay Co., Philadelphia 5, Penn.) of 30–60 mesh. The two outer rows in each plot and 3 feet at the end of each row were used as buffers. This allowed for movement of larvae from plot to plot, and also for spray drift from adjoining DDT-emulsion treated plots. Oviposition records were taken four times at weekly intervals, the counts being made only in the check plots. Twenty-five plants were examined in each plot on each occasion. The total number of eggs found on 100 plants on each date was: June 19, 17; June 26, 112; July 3, 71; and July 10, 16. The insecticides were applied on the last three dates. On the first date of application the plants were in the early to mid-whorl stage, on the second date they were in the late-whorl stage, and on the third date tasselling was 100% complete and about 15% of the silks were showing. Because of the small size of the plots, a type of hand shaker was devised for applying the materials, the openings being varied according to the percentage of toxicant. As far as possible each plant in each treatment was given the same amount of insecticide. Six plants were taken at random from the center four rows of each plot and dissected to find the borers. Also, in both 1956 and 1957, the number of marketable ears and the number of plants in each plot were recorded; a plant showing any injury was classified as infested whether an entry had been made into the plant or not.

In 1957, a 4-row pull-type applicator (Noble Manufacturing Company, Sac City, Iowa) was used for applying the granulated insecticides under conditions more nearly approaching actual field operations. This machine is so designed that granules placed in a large hopper feed slowly down a drop tube and fall directly into the whorls of the corn plants. A mechanism called a "fluted feed" governs the amount of material applied per acre. This machine was pulled at 3 miles per hour. Because the granulated insecticides were applied by a large machine, a single plot, 1/18 of an acre in area, larger than those used in 1956, was used for each treatment. There were six rows in each plot, and each row was 120 feet long. Endrin, heptachlor, and granulated DDT were used at lower dosages than in 1956. Dipterex (the agricultural formulation called Dylox) was included in the test because of its reputedly quick-killing action against insects, and for its low mammalian toxicity, as noted by Deichmann & Lampe (1955). Oviposition, which was lower in 1956, was recorded for 100 plants taken at random in the check plot on each of three occasions. The dates and the numbers of egg masses found were: June 18, 57; June 25, 53; and July 3, 6. Applications were made on the three above-mentioned dates. Plant growth was somewhat more advanced than in 1956. The first application was made when the plants were in the mid-whorl stage; the second when the plants were in the late-whorl stage with a few tassels showing; the plants showed 100% tassel and 100% silk at the time of the third application. The sampling area consisted of the center four rows of each plot, less 10 feet at the end of each row. The remaining portion was buffer area. Sampling tables (Kendall & Smith 1951) were used to select 24 plants from each plot for dissection. Records were made of the number of larvae per plant and also the number of

marketable ears and the percentage infested plants per treatment.

RESULTS AND DISCUSSION.—In 1956, all the treatments gave satisfactory percentages of marketable ears as well as reductions in numbers of borers (table 1); both endrin and heptachlor allowed significantly fewer larvae per plot than the other treatments. The toxaphene plots had significantly fewer borers than the plots of either DDT treatment, but the percentage of marketable ears produced in the toxaphene-treated plots was lower than for any other treatment. The percentage of marketable ears produced in plots treated with endrin or heptachlor was outstanding.

In 1957, the treated plots did not differ significantly in the average number of larvae per plot. There was no evidence of phytoxicity in any of the treated plots. The reduction in the amount of toxicant used per acre did not appreciably reduce the percentage of marketable ears. However, the infestation in 1957 was lower than in 1956, when only 2% of the ears in the untreated check were marketable, in comparison with 14% in 1957.

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Effect of Methyl Bromide and Hydrocyanic Acid Fumigation on the Germination of Wheat[1]

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ABSTRACT

Two varieties of wheat with a graded moisture range of 8, 10, 12, and 14% were fumigated with methyl bromide and hydrocyanic acid in 100-cubic-foot gas-tight chambers. Exposure periods, dosages of fumigant applied, and temperatures during fumigation were varied. One-half of the seed samples received one fumigation; the others were fumigated twice. Two series of germination tests were made, the first within 5 days following fumigation of seeds, and the second after 84 days of post-fumigation storage at 50° F.

Under the conditions of these studies, the germination of wheat was not affected by fumigation with hydrocyanic acid. Injury resulting from methyl bromide fumigations could be identified as retarded emergence and growth of roots and shoots in various degrees, ranging up to complete absence of seed germination.

The variables which may influence the germination of wheat fumigated with methyl bromide and their effects are summarized as follows:

Differences between the varieties of wheat may be attributed to seed quality rather than to true differences in varietal susceptibility to methyl bromide.

Increases in the moisture content of seeds contributed to decreases in germination following methyl bromide fumigation.

As exposure periods were shortened, increases in percentages of germination were observed.

Increases in temperature resulted in decreases in percentages of germination.

Percentages of germination decreased with an increase in dosages applied when other variables remained constant.

Results from germination tests suggests that repeat fumigation is a dependent variable as increased injury occurred after two fumigations with methyl bromide only in cases where injury was indicated after one fumigation.

No significant differences were observed in the percentages of germination and live seeds between tests made immediately after fumigation and those made after post-fumigation storage of seeds for 84 days.

Results obtained from germination tests illustrate the interdependence of dosage, temperature, period of exposure, and moisture content of seeds in contributing to seed injury from methyl bromide fumigation.

The problem of seed tolerance to fumigants is enlarged in programs designed to eradicate a storage pest such as the khapra beetle, *Trogoderma granarium* Everts, since heavy dosages and long exposures are necessary. In such a program, situations and circumstances contributing to reduction or failure of seed germination due to fumigation may be encountered more readily than in the usual disinfestation of grain.

It is the purpose of this paper to evaluate several factors which may influence the germination of wheat fumigated with methyl bromide or hydrocyanic acid. The studies reported were made under controlled conditions and proven techniques were followed. Wheat fumigated with hydrocyanic acid was not injured, but, in every instance, injury resulting from methyl bromide fumiga-

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tion may be attributed to a single factor or the interaction of a number of factors.

MATERIALS AND METHODS.—*Preparation of Seed Samples.*—Two varieties of wheat, Ramona 50 and Onas 54, representative of a wide range of hard and soft western white wheats were used. Moisture content (m.c.) of the seeds due to normal fluctuations in storage ranged from 8.5 to 10.3% in Ramona 50 and 9.5 to 11.3% in Onas 54 during the experimental period of approximately 18 months.

Obtaining wheat with a graded moisture range of 8, 10, 12, and 14% required careful adjustment of moisture and conditioning of seeds. The difficulties involved are apparent, but persistent drying, addition of water, or alternation of drying and addition of moisture resulted in wheat samples of the desired moisture content.

Seeds were dried in narrow wire-screen containers placed in a forced-air oven maintained at 125 to 130° F. This temperature did not affect the germination of wheat samples. The drying period ranged up to 3 hours at any one time, but never longer. The moisture content of seeds before drying and the desired moisture level indicated the length of time seeds were to be held in the air-oven. Water was added to increase the moisture content of seeds. After drying or addition of water, seeds were conditioned in air-tight polyethylene bags for 7 days at 50° F. Then seeds were blended and moisture tests were made to determine the need for additional moisture adjustments. Moisture content of seeds was determined by the air-oven method specified in the A.O.A.C. Official Methods of Analysis (Horwitz 1955).

When the moisture content of wheat had reached the desired level, each lot of seeds was divided into samples of equal size, approximately 100 grams per sample, and put into small rough-woven cotton bags. Seeds were removed from these bags only for germination tests. Each cloth sample-bag of wheat was placed in an air-tight polyethylene bag to prevent moisture changes. Prepared samples awaiting fumigation were held in storage at 50° F.

Fumigation of Seeds.—Two factors considered in this study, varietal differences and moisture content of seeds, were treated in the preceding section on preparation of seed samples. Additional factors under consideration concerned directly with the fumigation of seeds were: the exposure period, temperature during fumigation, dosage levels, and repeat fumigation.

Test samples were removed from storage and held at the desired fumigation temperature before removing air-tight polyethylene bags. Seeds of all four moisture levels were placed in slatted crates inside the fumigation chambers to be fumigated together. The crates permitted thorough circulation of gases.

Fumigation experiments were conducted in 100-cubic-foot gas-tight chambers with continuous circulation of air within the fumatorium. Temperatures were thermostatically controlled at 50, 70, and 90° ± 1° F., but no attempt was made to control humidity. Fumigations were replicated twice.

Two dosages of fumigant, one high and one low, were used throughout the study. Dosages were adjusted according to exposure periods and temperatures during fumigation. When the temperature was lowered, dosages usually were increased for an exposure period. Further, as the period of exposure was decreased, usually the dosages of fumigant were increased. An exposure period of 72 hours was selected for beginning tests. When seeds were injured at this exposure with the dosages applied, tests were made with shorter exposure periods.

Table 1.—Exposure periods, temperatures, and dosages of hydrocyanic acid and methyl bromide used for the fumigation of wheat seeds.

Fumigant	Exposure (Hours)	Temperature (° F.)	Dosage[a] Low	Dosage[a] High
Hydrocyanic acid	72	90	1.0	2.0
		70	1.0	2.5
		50	1.0	3.0
Methyl bromide	72	90	1.0	3.0
		70	1.5	3.0
		50	2.0	4.0
	48	90	1.5	4.0
		70	2.0	4.0
		50	2.5	5.0
	24	90	1.5	4.0
		70	2.0	4.0
		50	2.5	4.0
	8	90	2.0	5.0
		70	3.0	5.0
		50	3.0	6.0
	2	90	3.0	6.0
		70	3.0	6.0
		50	3.0	6.0

[a] Pounds per 1000 cubic feet.

The exposure periods, temperatures during fumigation, and dosages of fumigants applied are given in table 1. Concentrations of fumigants were not measured for each dosage or exposure interval. However, typical curves for the fall in concentrations of methyl bromide are given in figure 1. These show the actual concentration of methyl

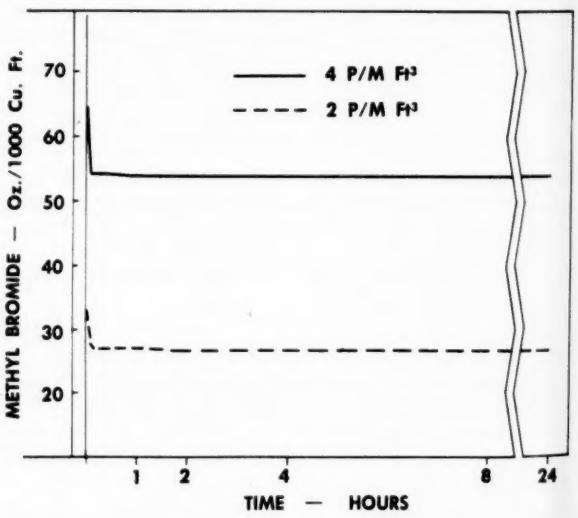

FIG. 1.—Relation between dosages of methyl bromide applied in 100-cubic-foot fumatorium and concentration of fumigant in gas phase at various sampling intervals.

bromide within the chamber with dosages of 2 and 4 pounds per 1000 cubic feet for a 24-hour exposure period at 70° F.

All seeds received an initial fumigation. One-half of the samples were fumigated a second time 21 days after the initial exposure to test the effect of repeat fumigation on the germination of wheat. After fumigation, seeds were aerated for 24 hours before repackaging in air-tight polyethylene bags. Undoubtedly slight moisture changes occurred during aeration of seeds, but no attempt was made to adjust moisture after the initial fumigation. Seed samples were held in post-fumigation storage at 50° F. and removed only for germination tests.

Germination Tests and Assessment of Results.—Two series of germination tests were made, the first within 5 days following fumigation of seeds, and the second after 84 days (12 weeks) in storage at 50° F. Seeds were germinated at 68° F. in folded-paper-towel substrata.

Germination tests and seedling interpretations were made in accordance with regular methods of Official Seed Analysts (Assoc. Off. Seed Analysts 1954) except seeds with obvious mechanical injury were not planted, and 100 seeds were used per test for each treatment replicate. Non-fumigated seeds served as control standards for comparison.

The percentage of live seeds (normal and abnormal seedlings) and the percentage germination (normal seedlings) were the two essential criteria recorded for comparison of results. (See p. 131, U. S. Dept. Agric. Agricultural Handbook No. 30, 1952, for a description of normal and abnormal seedlings.) The distinction made between live seeds and germinated seeds is important since fumigants may cause injury by retarding germination as well as destruction of germinative capacity. Cobb (1956) contends that methyl bromide fumigation injury to seed may be identified as retarded emergence and growth of roots and shoots in various degrees, with a possibility of increase in weak sprout percentages, ranging up to complete absence of seed germination.

RESULTS.—Since 3,456 germination tests (100 seeds per test) were made during this study, it is necessary to materially condense results and present only a summary of data essential to the understanding and discussion of factors investigated. Results from fumigated seeds are presented as the corrected percentages of germination and corrected percentages of live seeds. These are based on 100% and were calculated by extending Abbot's (1925) formula as follows:

$$CP = 100\% - \left(\frac{A - B}{A} \times 100\right)$$

CP = Corrected percentage germination (normal seedlings), or corrected percentage of live seeds (normal and abnormal seedlings).
A = Unfumigated control, per cent germination or per cent live seeds.
B = Fumigated seeds, per cent germination or per cent live seeds.

The actual per cent germination of control samples (576 germination tests for each variety of wheat) were tabulated and are summarized graphically in figure 2. The frequency of occurrence refers to the number of tests

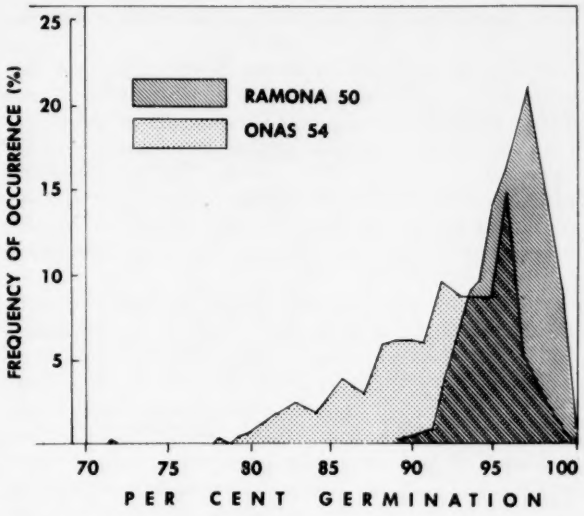

FIG. 2.—Frequency (expressed as a percentage of 576 germination tests) at which percentages of germination occurred in unfumigated control samples of Ramona 50 and Onas 54 wheat.

(expressed as a percentage of 576 germination tests) for a given percentage germination (per cent of normal seedlings in a test). Referring to figure 2, differences in seed quality of the two varieties of wheat are readily seen. Ramona 50 has a narrow range in the percentage germination of control samples, from 89 to 100%, with 97% germination occurring most frequently. Germinations in over 90% of Ramona 50 controls ranged from 92 to 99%. Germination in control samples of Onas 54 wheat ranged from 72 to 100%; however, the germination in 90% of the samples ranged from 85 to 98%. Other than quality of seeds, no explanation can be given for the erratic results from Onas 54 unfumigated control samples. Experimental processing and handling evidently did not contribute to this condition as it occurred inconsistently throughout the experimental period.

Hydrocyanic Acid Fumigation.—Exposure of two varieties of wheat with a graded moisture range of 8, 10, 12, and 14% to hydrocyanic acid fumigation for 72 hours at temperatures and dosages listed in table 1 did not result in significant reduction in the germinative capacity of seeds. For this reason, only a summary of results for the extremes in treatments are presented in table 2. It is ob-

Table 2.—Corrected percentage germination of two varieties of wheat after fumigation with high dosages of hydrocyanic acid for 72 hours at three temperatures.

Wheat Variety	Per Cent Moisture Content of Seeds	No. of Fumigations	Fumigation Temperature and Pounds per 1000 Cubic Feet: 90° F.—2.0	70° F.—2.5	50° F.—3.0
			Corrected Percentage Germination		
Ramona 50	8	1	100	100	99
		2	99	100	100
	14	1	99	96	98
		2	98	100	100
Onas 54	8	1	100	99	95
		2	100	100	100
	14	1	100	97	100
		2	97	100	98

vious from these data that further discussion is unnecessary.

Methyl Bromide Fumigation.—Results from the first series of germination tests (seeds planted within 5 days after fumigation) are presented graphically in figures 3 to 7 as the corrected percentages of germination and the corrected percentages of live seeds. The striking differences between treatments indicate that several variables considered in this study contributed to the effects obtained from methyl bromide fumigation. This is discussed more fully below.

DISCUSSION OF RESULTS FROM METHYL BROMIDE FUMIGATIONS.—The difficulties involved in separating factors for discussion and evaluation of their individual importance in relation to other variables are recognized. Some variables, for example—exposure period, temperature, and dosage, are considered together in fumigations for insect control due to the importance of their interactions. It is acknowledged that complete isolation of factors was possible by controlling all other variables. This, however, would have required extensive multiplication of the already large number of fumigation experiments and germination tests conducted without an extensive gain in information.

Seven factors were considered of primary importance in this study. These are listed and discussed separately; afterwards comments are made on the interaction of factors in contributing to the injury of wheat seed from methyl bromide fumigation.

It was stated previously that the percentages of live seeds and the percentages of germination were the essential criteria recorded for comparison of results. These percentages were corrected on a basis of 100% and the results shown graphically in figures 3 to 7. The percentages of dead seeds may be determined from the data by interpolation. Thus, three criteria are available for comparison of results—percentages of germination, live seeds, and dead seeds.

Varietal Differences.—It is possible to make 240 paired comparisons between Onas 54 and Ramona 50 wheat seeds in regard to their sensitivity to methyl bromide under various conditions from the data presented in figures 3 to 7. Approximately 75 pairs, slightly less than one-third of the observations, show differences of a varying degree between varieties. Only differences sufficiently significant to be apparent by visual inspection were considered. The differences observed favor Ramona 50 as being less sensitive to methyl bromide than Onas 54.

Differences between varieties in sensitivity to methyl bromide fumigation cannot be identified positively as true varietal differences. Rather, the data presented in figure 2 from germination tests of unfumigated control samples suggests considerable differences in seed quality. Although measurements were not made, seedlings of Ramona 50 were obviously more vigorous than those of Onas 54 in germination tests with unfumigated seeds.

Cobb (1956) mentions seed vigor as a possible important variable to consider. Later he (Cobb 1958) used poor quality feed corn in experiments with methyl bromide to test the effect of temperature because he expected differences to be more pronounced than with seeds of good quality. Results reported by Lubatti & Blackith (1957) from germination tests following fumigation with methyl bromide suggests that the main differences in the reaction of cereals to methyl bromide lie between the species, and that investigation of a wide range of varieties for each species is not an urgent task.

The natural differences in hardness of Onas 54 and Ramona 50 wheat suggested varying penetration of seeds by methyl bromide which might result in differences in germination between the varieties. However, from the data obtained in this study and the results of other workers, it must be concluded that differences observed between varieties of wheat, Onas 54 and Ramona 50, in susceptibility to methyl bromide may be due to the vigor or quality of the seeds.

Moisture Content of Seeds.—Results from germination tests with wheat seeds of a graded moisture range (8, 10, 12, and 14%) following fumigation with methyl bromide show a decrease in percentages of live seeds and germination with an increase in moisture content (figures 3 to 7). Similar results were obtained by Lindgren *et al.* (1955) with other species of seeds, and Cobb (1958) presents data in complete agreement.

Specific examples of a graded decrease in germination and live seeds which may be correlated directly with a graded increase in moisture are found in figures 3 C (two fumigations), 4 F (one fumigation), and 5 F (two fumigations). The critical moisture content appears to lie between 10 and 12% when other variables permit moisture to dominate. This is observed in varying degrees, but attention is directed to one specific example. In figure 3 F (one and two fumigations) there is a sharp decrease in the percentages of germination and live seeds at 12% moisture content. The critical moisture content, however, is dependent on other variables and may change according to conditions. In figures 4 A (two fumigations) and 6 B (two fumigations) the critical moisture content is found between 8 and 10%. Temperature, dosage, or repeat fumigation, alone or in combination, may have contributed to this condition. The effect of moisture content is completely dominated in some tests (figures 3 B and 4 B), and in others injury does not occur. This suggests that visible moisture effects are often the result of interactions with other variables and cannot always be isolated conveniently.

Period of Exposure to Fumigant.—The interaction of so many variables contribute to seed injury from methyl bromide fumigation that it becomes increasingly difficult to attribute much emphasis to an individual factor. However, a view of all the results presented (figures 3 to 7) is sufficient to show the importance of exposure. As periods of exposure are decreased, germination increases. This conclusion is emphasized further by comparing results from tests with seed injury where only the exposures varied, other factors remained constant. Such comparisons can be made between results presented in the following figures: 4 A and 5 A, 4 B and 5 B, 4 C and 5 C, 4 D and 5 D, 4 E and 5 E, 6 C and 7 C.

Temperature During Fumigation.—Where seeds were affected by methyl bromide fumigation, increases in temperature resulted in decreasing percentages of germination and live seeds (figures 3 to 7). This was anticipated as dosages applied for insect control must be increased with lowering of temperatures. As seen in table 1, a logical selection of dosages was made with due consideration

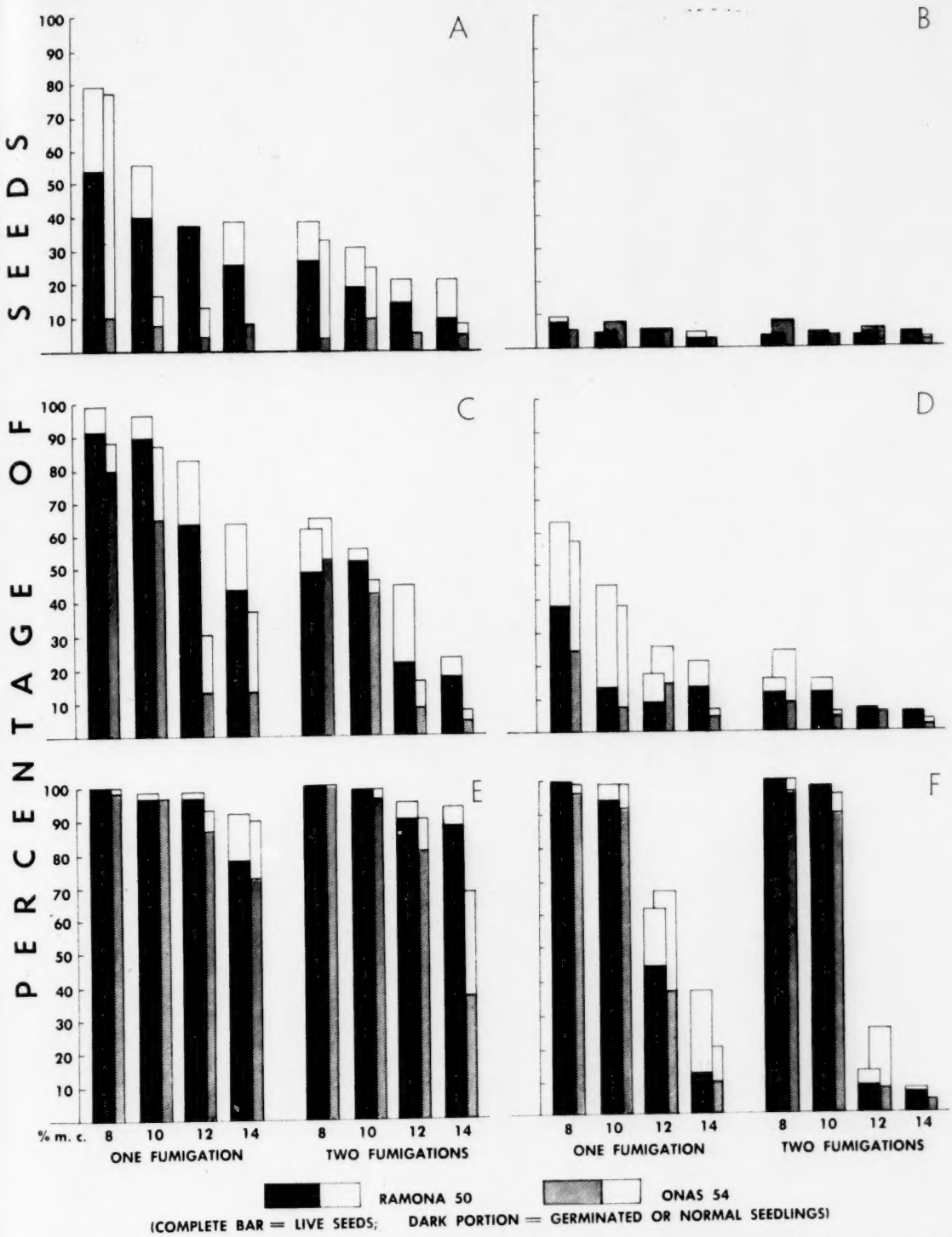

FIG. 3.—Corrected percentages of germination and live seeds for two varieties of wheat after one and two methyl bromide fumigations for 72 hours at various temperatures and dosages: (A) 90° F.—1.0 pound per 1000 cubic feet, (B) 90° F.—3.0 pounds per 1000 cubic feet, (C) 70° F.—1.5 pounds per 1000 cubic feet, (D) 70° F.—3.0 pounds per 1000 cubic feet, (E) 50° F.—2.0 pounds per 1000 cubic feet, and (F) 50° F.—4.0 pounds per 1000 cubic feet.

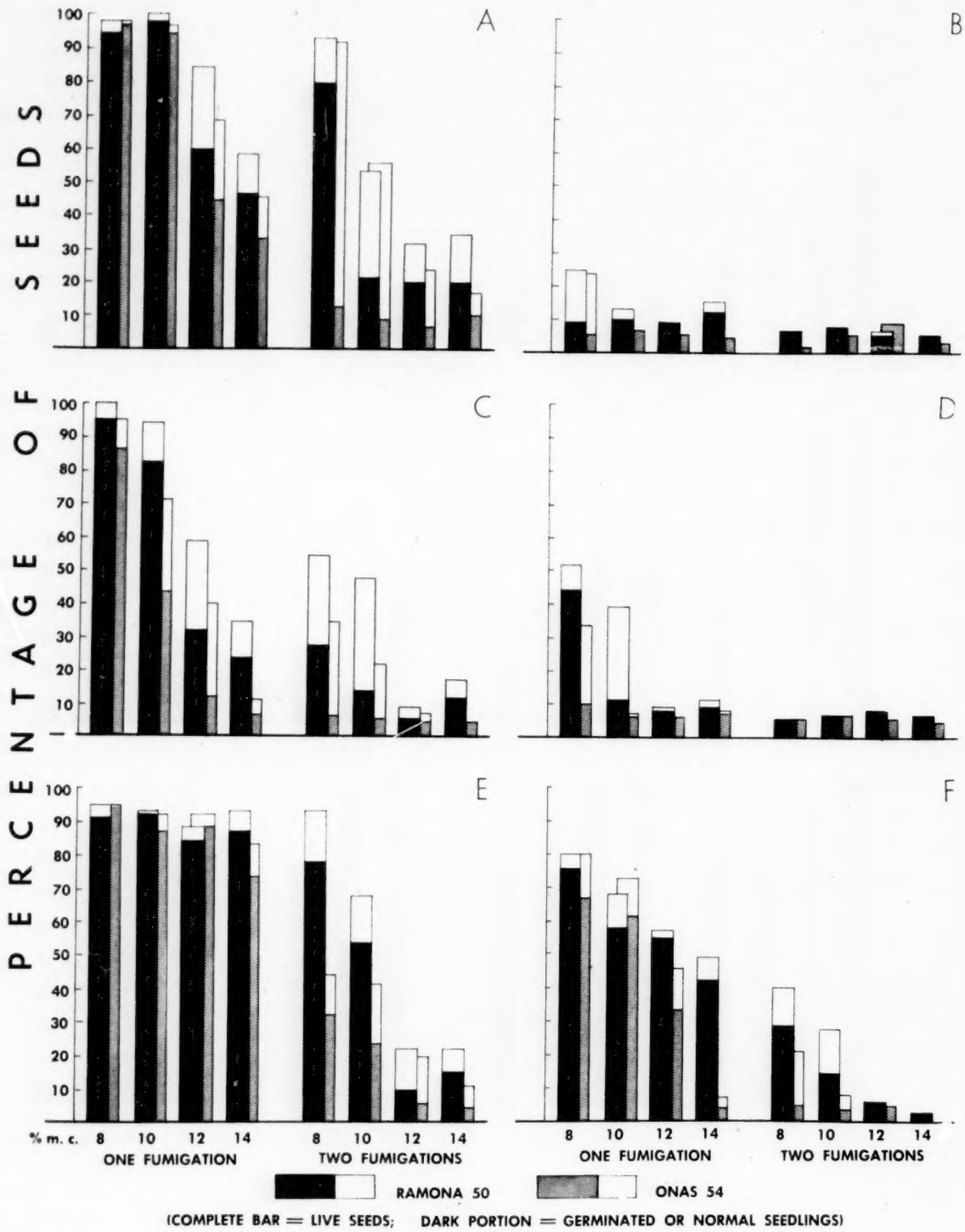

FIG. 4.—Corrected percentages of germination and live seeds for two varieties of wheat after one and two methyl bromide fumigations for 48 hours at various temperatures and dosages: (A) 90° F.—1.5 pounds per 1000 cubic feet, (B) 90° F.—4.0 pounds per 1000 cubic feet, (C) 70° F.—2.0 pounds per 1000 cubic feet, (D) 70° F.—4.0 pounds per 1000 cubic feet, (E) 50° F.—2.5 pounds per 1000 cubic feet, and (F) 50° F.—5.0 pounds per 1000 cubic feet.

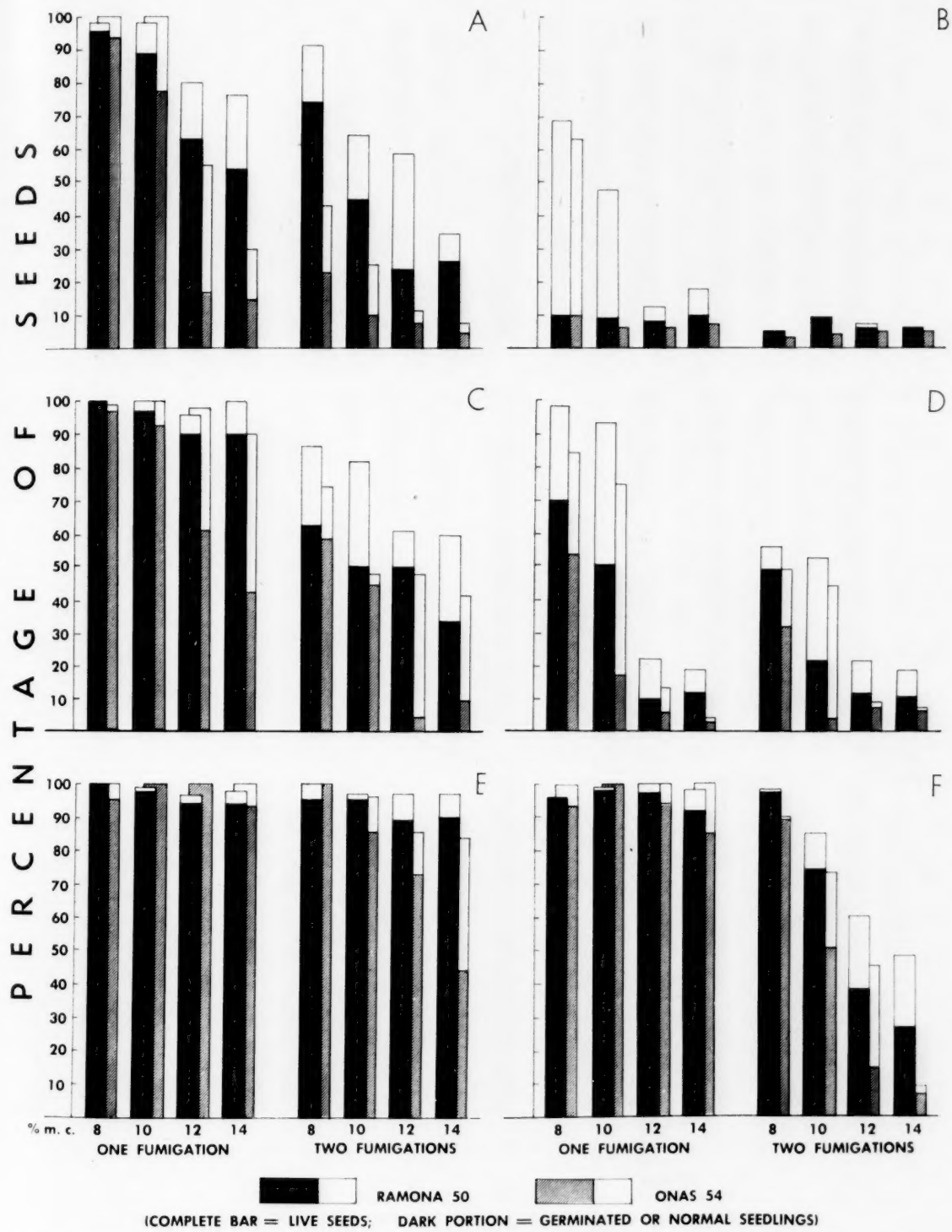

FIG. 5.—Corrected percentages of germination and live seeds for two varieties of wheat after one and two methyl bromide fumigations for 24 hours at various temperatures and dosages: (A) 90° F.—1.5 pounds per 1000 cubic feet, (B) 90° F.—4.0 pounds per 1000 cubic feet, (C) 70° F.—2.0 pounds per 1000 cubic feet, (D) 70° F.—4.0 pounds per 1000 cubic feet, (E) 50° F.—2.5 pounds per 1000 cubic feet, and (F) 50° F.—4.0 pounds per 1000 cubic feet.

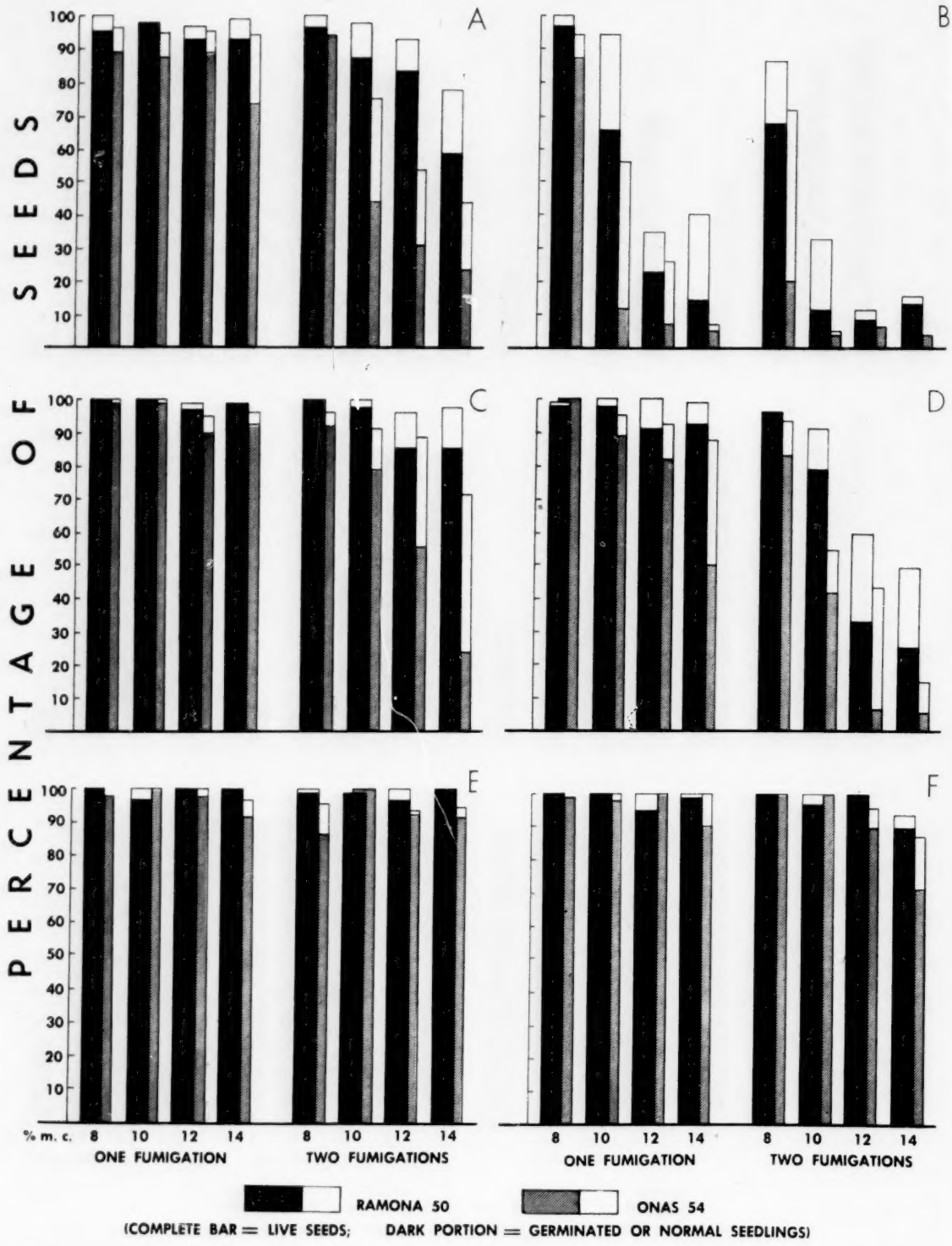

FIG. 6.—Corrected percentages of germination and live seeds for two varieties of wheat after one and two methyl bromide fumigations for 8 hours at various temperatures and dosages: (A) 90° F.—2.0 pounds per 1000 cubic feet, (B) 90° F.—5.0 pounds per 1000 cubic feet, (C) 70° F.—3.0 pounds per 1000 cubic feet, (D) 70° F.—5.0 pounds per 1000 cubic feet, (E) 50° F.—3.0 pounds per 1000 cubic feet, and (F) 50° F.—6.0 pounds per 1000 cubic feet.

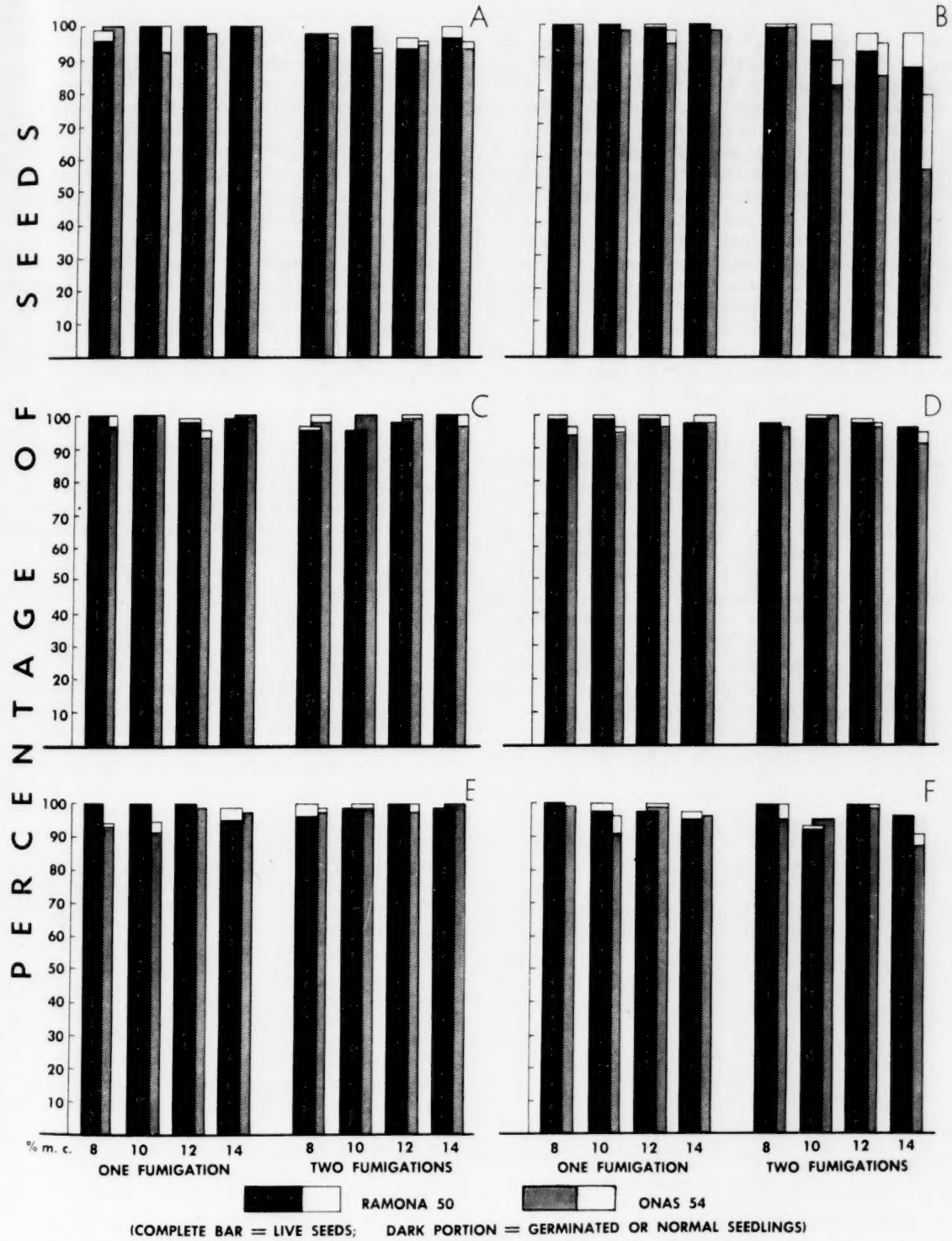

FIG. 7.—Corrected percentages of germination and live seeds for two varieties of wheat after one and two methyl bromide fumigations for 2 hours at various temperatures and dosages: (A) 90° F.—3.0 pounds per 1000 cubic feet, (B) 90° F.—6.0 pounds per 1000 cubic feet, (C) 70° F.—3.0 pounds per 1000 cubic feet, (D) 70° F.—6.0 pounds per 1000 cubic feet, (E) 50° F.—3.0 pounds per 1000 cubic feet, and (F) 50° F.—6.0 pounds per 1000 cubic feet.

for the interaction of temperature and dosage.

An effort was made, however, to allow opportunities for the isolation of temperature from other variables. Results are given for fumigations at 4.0 pounds of methyl bromide per 1000 cubic feet in figure 5 B, D, and F. From these results, it is apparent that as the temperature is lowered from 90° F. (figure 5 B) to 70° F. (figure 5 D), and to 50° F. (figure 5 F) the percentages of germination and live seeds increases accordingly. Similar comparisons can be made between results presented in figures 3 B and D, 4 B and D, 6 B and D, and 6 C and E.

Dosage of Fumigant.—Since eradication programs require somewhat higher than normal dosages of fumigant, two dosage levels (one low and one high) were considered for each set of conditions involved in this study. Paired comparisons may be made between dosage levels from the results presented in figures 3 to 7 by horizontal examination of the data, from left to right, in each figure. The percentages of germination and live wheat seeds decreases with an increase in dosage when other variables remain constant. The interdependence of dosage and temperature, however, can be seen by a vertical examination of results in figures 3 to 7. It is evident that an increase in dosage with a decrease in temperature does not result in a decrease in the percentages of germination and live seeds.

Repeat Fumigation.—High dosages of methyl bromide and long exposure periods are employed in eradication programs (for example, khapra beetle eradication) so that repeat fumigations are not required except under unusual circumstances. Seed sanitation, however, usually requires more than one fumigation to prevent injury from insects. Thus, the effect of repeat fumigation on the germination of seeds is an important factor to consider.

Results given in figures 3 to 7 suggest that repeat fumigation is a dependent variable as increased injury occurred with two fumigations only in cases where injury was indicated after one fumigation. Presumably, when seeds have a predisposition to injury after one fumigation, the injurious effect of methyl bromide is greatly enlarged by two fumigations.

Post-fumigation Storage.—A second series of germination tests was made after post-fumigation storage of seeds for 84 days (12 weeks) at 50° F. No significant differences were observed in the percentages of germination and live seeds between tests made immediately (within 5 days) after fumigation and those made 84 days later. For this reason, results from the latter tests are not shown as it would be a mere duplication of results presented in figures 3 to 7. These results concur with those of Cobb (1958) and agree with his conclusion that fumigation injury occurred while seeds were in contact with the fumigant, that fumigation injury was irreversible, and seeds uninjured during fumigation do not show injury later, except after repeat fumigation when seeds are predisposed to injury.

Interaction of Variables.—Dosage, temperature, and period of exposure are inseparable variables in considering fumigations for insect control. Results presented in figures 3 to 7 illustrate the interdependence of these factors and another, the moisture content of seeds, in contributing to seed injury from methyl bromide fumigation.

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The Effect of Three *p*-Chlorophenyl Compounds on the Biology of the House Fly[1]

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Abstract

When 1,1,*bis* (*p*-chlorophenyl) chloromethane (DBC), 1,1,*bis* (*p*-chlorophenyl ethanol (DMC) and 1,1,*bis* (*p*-chlorophenyl ethnyl carbinol (EC), which synergize the contact effect of DDT on adult house flies (*Musca domestica* L.), were added in amounts of from 1.3 to 130 parts per million to the milk used to moisten a "cellu-cotton" substratum in which house fly larvae were reared there was some mortality of early instar larvae, but those that survived grew with normal rapidity, reached normal weight, and gave rise to normal pupae and adults except that surviving third instar larvae were more susceptible to contact with DDT than those from untreated media. Pupae and adults showed no greater susceptibility. Pupation was slowed down but fecundity was unaffected. The eggs laid were of normal viability and adult longevity was normal.

Several chemicals synergistic with pyrethrum have been shown to inhibit the development of the house fly (*Musca domestica* L.) when incorporated into the standard Chemical Specialties Manufacturers Association larval rearing medium (Mitlin & Konecky 1955, Konecky & Mitlin, 1955, Mitlin 1956). Ascher (1957) has reported the prevention of oviposition in house flies following exposure to residues of several *p*-chlorophenyl compounds and Mitlin *et al.* (1957) the prevention of ovarian development with resultant sterility in female flies after treatment with aminopterin, a nitrogen mustard, and colchicine. Reference was made by Hagley & Morrison (1958) to studies then in progress on the effects of incorporating 1,1-*bis* (*p*-chlorophenyl) chloromethane (DBC), 1,1-*bis* (*p*-chlorophenyl) ethanol (DMC), and 1,1,-*bis* (*p*-chlorophenyl) ethyl carbinol (EC) into house fly rearing media. The effects of these chemicals on early larval mortality, larval susceptibility to DDT, average larval weight, rate of pupation, pupal susceptibility to DDT, adult suceptibility, the fecundity of resultant adults, the viability of eggs from such adults and the longevity of such adults are reported here.

Procedure.—Flies were reared on a milk medium according to the method of Fisher & Morrison (1952), except that for this work large test tubes, were substituted for pint milk bottles and the amount of medium correspondingly reduced. The chemicals under test were incorporated into the rearing medium by adding to each 30 ml. of milk, before it was used to wet the "cellu-cotton," 0.2 ml. of benzol with the desired amount of chemical dissolved in it. Milk and benzol solution were hand shaken into a homogeneous emulsion before they were added to the substratum. Fifty eggs were placed in each tube. Concentrations of the chemical were recorded as percentages (weight on volume) of the milk. Larvae were removed when they reached the third instar and placed in dry bran as a pupation medium. The susceptibility of third instar larvae to DDT was tested by exposure to DDT impregnated filter paper for a period of time calculated to kill a small percentage of larvae from non-treated media followed by removal to a dry medium The mortality was noted after 24 to 48 hours.

Table 1.—Effects of *p*-chlorophenyl compounds in house fly rearing medium on larval survival.[a]

Treatment	No. Third Instar Larvae[b]	Per Cent Larva Pupated	Average Weight of Pupae[c] (mg.)	Per Cent Adult Emergence
None	31	88	16.50	82
Benzol 0.2 ml.[d]	25	79	15.60	78
DBC (130 p.p.m.)	16	77	15.46	73
DMC (130 p.p.m.)	11	75	16.33	65
EC (130 p.p.m.)	10	63	13.16	56
DBC (13 p.p.m.)	19	77	13.30	73
DBC (13 p.p.m.)	17	82	11.83	71
EC (13 p.p.m.)	16	59	11.66	69
DBC (1.3 p.p.m.)	23	72	12.45	78
DMC (1.3 p.p.m.)	25	72	12.75	71
EC (1.3 p.p.m.)	21	78	12.50	62

[a] Figures are averages from four tests with 50 eggs each.
[b] F = 5.760—L.S.D. = 3.61—95% fiducial limits.
[c] F = 2.889—L.S.D. = 3.11—95% fiducial limits.
[d] All other chemicals were added in this amount of benzol, and amounts expressed as parts per million (p.p.m.) in milk.

Results.—Incorporation of the synergists resulted in some mortality of early instar larvae (table 1) but those that survived grew with normal rapidity, reached normal weight (table 2) and showed no impairment of vigour or activity other than an increased susceptibility to DDT.

The rate of pupation was, however, slowed down (table 4) but the resultant pupae and adults were normally susceptible to DDT (table 5).

The effects of the treated media on the resultant adult flies are summarized in table 6, 7 and 8. The (DBC) treatment resulted in an increase in the number of eggs laid by adult female flies. Afifi & Knutson (1956), and Ouye & Knutson (1957) report a similar increase in the fecundity of adult female flies from media treated with sublethal dosages of dieldrin and malathion, respectively.

None of the other chemicals had any effect on the

[1] Accepted for publication July 21, 1958.

Table 2.—The weight of mature third instar larvae recorded from treated and untreated larval media.[a,b]

Treatment of Medium (130 p.p.m.)	Average Weight in μgm.
None	20.15
Benzene (0.2 ml.)	20.30
DBC	18.87
DMC	18.60
EC	19.69

[a] Each figure represents the average of 10 larvae weighed from six individual treatments.
[b] F = 0.231, not significant at 95% fiducial limits.

Table 3.—The effect of exposure to DDT-residues on larvae reared in synergist-treated media.

Treatmeet	Per Cent[a] Larval Mortality	Per Cent Larvae	Average Pupal Weight	Per Cent Adults
None	14[b]	86[c]	13	76
Benzol (0.2 ml.)	38	68	12	65
DBC (130 p.p.m.)	58	44	10	46
DMC (130 p.p.m.)	48	50	11	30
EC (130 p.p.m.)	54	46	11	54

[a] Based on five replicates of 10 larvae each for each treatment.
[b] F=3.19—L.S.D.=3.19—95% fiducial limits.
[c] F=3.04—L.S.D.=2.946—95% fiducial limits.

Table 4.—The effect of three *p*-chlorophenyl compounds on the rate of pupation in the house fly.[a]

Treatment	Per Cent of Larvae Pupating on Day Shown[b] 1st	2nd	3rd	4th
None	35.1	27.6	9.2	6.9[c]
Benzol (0.2 ml.)	23.1	30.1	17.3	16.7[c]
DBC (130 p.p.m.)	14.3	28.6	42.9	
DMC (130 p.p.m.)	—	50.0	37.5	
EC (130 p.p.m.)	—	—	88.9	
DBC (13 p.p.m.)	11.1	25.4	25.2	
DMC (13 p.p.m.)	2.4	20.2	49.2	
EC (13 p.p.m.)	5.3	15.1	32.0	
DBC (1.3 p.p.m.)	32.6	23.0	16.0	
DMC (1.3 p.p.m.)	24.1	20.2	16.2	
EC (1.3 p.p.m.)	29.9	21.6	38.7	

[a] Each figure represents the average of four individual treatments.
[b] The first day is the first one on which any larvae entered the pupal stage, the second, third and fourth are successive thereafter.
[c] Observed in a single rearing only.

Table 5.—The effect of 0.70 microgram of DDT applied topically to individual pupae and adults from larvae reared in synergist-treated media.[a,b]

Treatment of Media	No. Pupae Treated	Per Cent Adult Emergence[c]	No. Adults Treated	Per Cent Adult Mortality[d]
None	110	87.9	91	42.6
Benzene	95	77.2	78	28.8
DBC	93	73.4	76	19.3
DMC	85	70.4	84	46.3
EC	99	66.5	80	55.3

[a] 1.3 p.p.m. of the synergists in the milk used in the rearing medium.
[b] Pupae and adults collected from three treatments. Only male adult flies were used.
[c] F=0.578, not significant.
[d] F=0.122, not significant.

fecundity of resultant female adults, and the fertility of eggs obtained was not affected by any treatment (table 7). Neither was the longevity of resultant flies affected (table 8).

Table 6.—The effect of three *p*-chlorophenyl compounds in the rearing medium on the fecundity of resultant female flies.[a,b]

Treatment of Medium[c]	Average Number of Eggs per Egg mass	Female
None	100	120
Benzene	81	123
DBC	70	143
DMC	82	120
EC	82	133

[a] Each figure represents the average of three individual treatments.
[b] F=12.564, L.S.D.=21.43—95% fiducial limits.
[c] 1.3 p.p.m. of chemicals used in the milk in the nedia.

Table 7.—Percentage hatch out of 80 eggs per treatment obtained from resultant adult female flies.[a]

Treatment of Medium[b]	Percentage Hatch[c]
None	77.5
Benzene	66.3
DBC	66.3
DMC	63.8
EC	65.0

[a] 20 eggs were used from each of four individual treatments.
[b] 13 p.p.m. of chemicals used in milk.
[c] F=2.36 not significant at 95% fiducial limits.

Table 8.—The length of life of 30 adult flies reared from each treated larval medium.

Treatment of Medium[a]	Longevity (days)[b,c]
None	11.5
Benzene	13.0
DBC	11.0
DMC	12.5
EC	13.0

[a] 130 p.p.m. of chemical used.
[b] Adult flies from three individual treatments observed. Length of life based on average of shortest and longest life span.
[c] F=0.54, not significant at 95% fiducial limits.

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Cultural and Chemical Control of the Lesser Cornstalk Borer in Southern California[1]

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Abstract

The lesser cornstalk borer, *Elasmopalpus lignosellus* (Zell.), is a pest of corn, sorghum, beans, grain, and a few other agricultural crops primarily in the southeastern desert areas of southern California but occasionally in southern coastal areas as well. Most weeds belonging to the grass family are important hosts in maintaining populations. Injury in California is confined almost entirely to the tender seedling stages of plant growth and arises primarily from migrating larvae resident within the field on miscellaneous hosts at planting time rather than from freshly deposited eggs. The most effective cultural control consists of destroying infested host plants within the field some weeks prior to planting. In the case of sorghums, which are often planted flat for flood irrigation, a well-timed irrigation decimates the population sufficiently that usually satisfactory plant stands are assured.

Successful insecticidal control is predicated upon preventive applications. Endrin, aldrin, heptachlor, and dieldrin were the most effective of several insecticides investigated. There was little difference in effectiveness between granulated or spray formulations. Applications made at time of plant emergence were slightly superior to those made at planting time under the conditions of these experiments. Insecticides were applied in a narrow band along the seed furrow or row of seedling plants. Results with insecticides were satisfactory when the seeds were planted in beds at about a 40-inch spacing, but when the seed was drilled into the soil in a flat planting with a row spacing of only a few inches, a similar amount of toxicant per acre did not give satisfactory plant protection.

The lesser cornstalk borer, *Elasmopalpus lignosellus* (Zell.), has been recognized as a pest across the southern part of the United States for many years. According to Luginbill & Ainslie (1917) it is limited in distribution to the Western Hemisphere, extending throughout much of South America, southern North America, and the West Indies. In California, the Imperial, Coachella, and Palo Verde valleys of the Colorado Desert are the only areas where economic damage has been noted consistently. At times damage occurs in coastal San Diego County.

Although this species was described by Zeller in 1848, it was not considered of economic importance until 1881 (Riley 1882). Since then a number of papers have appeared giving data on hosts, damage, control measures, etc. The most detailed and comprehensive life history study was made by Luginbill & Ainslie (1917).

Wild and Cultivated Host Plants.—Reports in the literature show that the lesser cornstalk borer attacks a rather wide range of host plants. In California, the most important cultivated hosts are corn, sorghums, and different varieties of beans. Occasionlly, infested pea plants have been found. Some hosts such as small grains (barley, oats, etc.) are planted too early in the year to undergo heavy attack, as in most cases populations of this insect are too low to cause serious injury until nearly midsummer and fall. Rarely, larvae will cause some damage to seedling cotton and ornamentals, particularly garden stock.

Important weed hosts in California are nut grass, water grass, Johnson grass, crab grass, wild oats, and volunteer grains. Larvae have been found attacking Bermuda grass, but it is not considered a good host. Foxtail is listed as a host (Isely & Miner 1944) but does not occur abundantly in areas of California where this pest is important. Hosts listed by Luginbill & Ainslie (1917) include beans, cowpeas, peanuts, corn, crab grass, chufa, Japanese cane, sugar cane, sorghum, milo, Johnson grass, wheat, and turnips. Other plants attacked are strawberries (Isely & Miner 1944), peppers (Dempsey & Brantley 1953), and gladiolus (Kelsheimer 1955). Undoubtedly other plant species are hosts of this insect. As noted by Luginbill & Ainslie (1917) and Isely & Miner (1944), the larvae have a wide host range, but show a decided preference for members of the grass family.

Damage.—Injury caused by the larvae of this species has been adequately described by a number of entomological workers. In view of this, only a brief description is included here. As the common name implies, the larva attacks the plants at or just below the soil surface, and if the plant is sufficiently tender it bores into or tunnels within the stem. Small plants thus injured soon wilt and often die. The larva thereupon leaves the wilted plant and migrates to another; a single larva may thus destroy a number of seedling plants. On older plants, feeding damage may go unnoticed, as the larvae are usually unable to penetrate into the stem and are forced into feeding on surface tissues. This in itself may not be seen by the grower, but in rare cases the stem may be weakened to the point where a strong wind will break the plant stem at the point of heaviest external feeding. This is most frequently noticed in the case of blackeye beans but is sometimes found in other crops as well. In California serious damage is confined to seedling plants in nearly all cases, although a few reports of damage to mature pole-bean plants are on record.

A silken tube covered with soil particles is usually found at the point of entry into the plant. In most cases, the bluish-green, active larva is found in this tube. Occasionally, however, it may be found within the stem or may be seen wandering over the soil surface apparently in search of a suitable host plant. As noted by Isely &

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Miner (1944), the larvae found attacking seedling plants are usually in the later stages of development, indicating that in most cases they began their development on another host.

CULTURAL CONTROL.—Luginbill & Ainslie (1917) recommended destruction of all stages of this insect occurring in plant residue during the winter period. Isely & Miner (1944) pointed out, however, that there are several generations a year and it is entirely possible that a large population will develop by midsummer regardless of the number of overwintering stages destroyed. Accordingly, the latter authors found that complete destruction or removal of all living plant residue in fields some weeks before planting was the most promising method of preventing serious outbreaks. This procedure has been moderately successful in California when carried out carefully, as normally only plants in the seedling stage of development are damaged seriously. The success of this method is predicated on the fact that partly grown larvae are no longer present to attack seedling plants, and larvae hatching from eggs laid after the seedling plants are established do not have time to develop to destructive size before the plants have passed the most susceptible stage of growth. Cultivating out infested weed hosts just before or after planting a susceptible crop can be disastrous, however, as it forces virtually the entire resident population to feed upon the seedling plants. For this reason, many crops, even those such as cotton, which is not normally considered a host, are quickly and often heavily attacked.

In California, successful control of the larvae has been obtained by carefully timed irrigation, applied in one of two ways. In the case of sorghum, seeds are often drilled into the soil in a flat planting for flood-irrigation (similar to that used in alfalfa hay plantings) as opposed to planting in beds for furrow-irrigation. In this way, when a serious infestation of lesser cornstalk borer larvae is noticed, it is possible to decimate the population by flooding the area between the borders with the normal flood-irrigation. Although not all the larvae are killed, sufficient protection is obtained to insure a satisfactory plant stand in most cases.

A second method in which irrigation water is utilized to stop an infestation has been only partly investigated to date, but on light sandy soils, at least, it offers some promise of success. According to this method, seeds of susceptible crops are planted in the bottom of the irrigation furrow. If the seedling plants later become seriously infested, the furrow is filled with water. The area between the plant rows may be furrowed out at a later date for irrigation when the plants are larger.

CHEMICAL CONTROL.—Control measures other than those utilizing cultural methods have been considered promising only in recent years. Wilson & Kelsheimer (1955) and Kelsheimer (1955) reported that chlordane was effective when applied as a 5% dust at a rate of 25 pounds per acre or as a spray applied at the rate of 1.5 to 2.0 pounds of toxicant per acre. These authors specified that to be successful the insecticide must be used as a preventive measure, for after the larvae are once established in the plant they cannot be successfully contacted with the chemical. Experimental work in California substantiated this observation, and several insecticides related chemically to chlordane were found to be effective.

Several field experiments were conducted before effective insecticides and the proper timing of applications were discovered. Only the more indicative experiments are reported here. Materials tested previously and not considered particularly promising were toxaphene, DDT, toxaphene-DDT combinations, and parathion. Results of tests in which Thimet and Bayer 19639 (Di-Syston) were used unsucessfully as seed coats or as granulated formulations incorporated with sorghum or soybean seed, were reported in an earlier paper (Reynolds *et al.* 1957).

Experiment No. 1.—This experiment was undertaken on August 24, 1956, on soybeans (Lee variety) in the Imperial Valley. Each treatment was replicated four times and each plot was one row (40 inches) wide by 50 feet long. All treatments were made immediately after planting except for one spray treatment and one treatment with granules, which were made just after plant emergence. All materials were applied in a band 2 to 4 inches wide along the top of the seed furrow. Granulated materials were formulated on either Attaclay or vermiculite and applied by hand. Sprays were applied with a hand-compression sprayer using one nozzle per row. All the treatments made immediately after planting, except one, were scratched lightly into the soil surface.

Treatments were evaluated on September 18, when the plants were 3 to 5 inches tall, by counting the number of plants killed or injured by the lesser cornstalk borer. As shown by the results of this experiment (table 1) all treatments gave good control of the lesser cornstalk borer and were significantly better than the check. There were no significant differences between any of the various treatments and methods or times of application. Apparently, the rates of toxicant applied per acre were too high to show differences between treatments.

Experiment No. 2.—This experiment was undertaken on June 14, 1957, on sorghum (Imperial Kaffir variety) in the Imperial Valley. Each treatment was replicated four

Table 1.—Results of an experiment (No. 1) in which several insecticides and times and methods of application were compared for control of the lesser cornstalk borer on soybeans planted August 24, 1956, Imperial Valley, California.

MATERIAL, FORMULATION, AND APPLICATION[a]	AMOUNT PER ACRE	POUNDS TOXICANT PER ACRE	NO. OF PLANTS INJURED OR KILLED IN 200 FEET OF ROW[b]
Aldrin, 20% granule on vermiculite	10 lb.	2	14[c]
Dieldrin, 20% granule on vermiculite	10 lb.	2	17[c]
Dieldrin, 20% granule on vermiculite[d]	10 lb.	2	3
Dieldrin, 20% granule on vermiculite[e]	10 lb.	2	4
Endrin, 8% granule on vermiculite	25 lb.	2	2
Heptachlor, 20% granule on vermiculite	10 lb.	2	4
Heptachlor, 10% granule on vermiculite	20 lb.	2	1
Heptachlor, 5% granule on vermiculite	40 lb.	2	3
Heptachlor, 20% granule on vermiculite	40 lb.	8	5
Heptachlor, 10% granule on vermiculite	40 lb.	4	3
Heptachlor, 10% granule on Attaclay	20 lb.	2	2
Dieldrin spray	41 gal.	2.5	2
Dieldrin spray[d,e]	41 gal.	2.5	6
Check	—	—	73
L.S.D. at 5% level			20

[a] Applied in band 2 to 4 inches wide along seed row. Unless otherwise specified, application was made immediately after planting by scratching into soil surface.

[b] On September 18 when the plants were 3 to 5 inches tall.

[c] High count may be due to error in application of treatment. In the case of aldrin, 12 out of the 14 injured plants, and in the case of dieldrin, 15 out of the 17 injured plants, were found in one replicate.

[d] Material applied to soil surface.

[e] Treatment applied immediately after plant emergence.

times, and each plot was 1 row (40 inches) wide by 100 feet long. All treatments were applied immediately after planting. Granulated treatments were formulated on Attaclay, except one, which was on vermiculite. Materials were applied with a very small rotary hand duster along and on top of the seed furrow in a band 3 to 5 inches wide. The seeds were planted in dry, loose soil composed mostly of small clods. Irrigation followed immediately after planting.

Treatments were evaluated on July 2, when the plants were several inches high, by counting the number of plants killed or injured by the lesser cornstalk borer. As shown by the results of this experiment (table 2) all the treatments gave fair control of the lesser cornstalk borer, in each case being significantly better than the check, except for the lowest dosage of dieldrin. There was no difference in effectiveness between dieldrin, heptachlor, and aldrin. An irrigation immediately following planting solidified the loose clod mulch into which many of the granules had fallen and quite possibly made some of the insecticide inaccessible to the larvae.

Experiment No. 3.—This experiment was undertaken in the same field and planting as No. 2. Instead of treating immediately after planting, however, applications were made just after the plants had emerged, when they were 0.5 to 2.0 inches tall. Plot size, the number of replications, and the method of applying the granules were the same as in experiment No. 2. Sprays were also included in this test, however, and were applied by a hand-compression sprayer. In addition to the other materials, endrin and Thiodan were used.

The results obtained in experiment No. 3 (table 2) show that several treatments gave excellent control but that two were barely significantly better than the check. Endrin spray (9 ounces per acre), endrin granules (8 ounces), dieldrin granules (16 ounces), dieldrin spray (7.6 ounces), and aldrin granules (on both Attaclay and vermiculite at 8 ounces) were significantly more effective than Thiodan and dieldrin spray applied at the rate of 19 and 4 ounces per acre, respectively. Results obtained with other dosages of dieldrin and heptachlor granules were intermediate, being neither significantly poorer nor better than the most or least effective treatments.

Miscellaneous Experiments.—Of the several other experiments undertaken in this study, one is of particular interest. In this case, sorghum was planted flat for flood-type irrigation as contrasted to the previous tests in which the seed was planted on beds for furrow-type irrigation. In flat plantings the sorghum seed is drilled into the soil with a grain planter with the row spacing usually about 6 to 7 inches apart.

In the experiment in question, granulated formulations of heptachlor, dieldrin, and endrin were applied at the rate of 2 pounds of toxicant per acre, and Thimet and Bayer 19639 were applied at the rate of 1 pound per acre. The materials were spread along the seed furrows and lightly scratched into the soil. All the treatments gave some protection from attack by the larvae of the lesser cornstalk borer, but the controls were not satisfactory. It appeared from these results that on flat plantings such as those described, the insecticide was spread too thinly along the closely spaced rows for good control as compared with the higher concentration of insecticide on the more widely spaced row plantings for furrow irrigation even though the total toxicant per acre may be the same. In cases such as these, a well-timed flood irrigation is desirable even though complete protection is not obtained

Table 2.—Results of two tests in the same field for control of the lesser cornstalk borer on sorghum. Planted June 25, 1957. Imperial Valley, California.

Material and Formulation	Ounces Toxicant per Acre[a]	No. of Plants Damaged or Killed in 400 Feet of Row	
		June 25	July 2
Applied immediately after planting just before irrigation (Experiment No. 2)			
Dieldrin, 2% granule on Attaclay	16.0	2	54
Dieldrin, 2% granule on Attaclay	8.0	5	86
Dieldrin, 2% granule on Attaclay	4.0	3	38
Dieldrin, 2% granule on Attaclay	2.6	2	49
Dieldrin, 2% granule on Attaclay	1.4	17	127
Heptachlor, 2.5% granule on Attaclay	8.0	1	65
Aldrin, 5% granule on Attaclay	8.0	0	69
Aldrin, 5% granule on vermiculite	8.0	0	38
Check	—	25	177
L.S.D. 5% level			74
Applied June 25 when the plants were 0.5 to 2.0 inches high (Experiment No. 3)			
Dieldrin, 2% granule on Attaclay	16.0		4
Dieldrin, 2% granule on Attaclay	8.0		56
Dieldrin, 2% granule on Attaclay	4.0		41
Endrin, 8% granule on vermiculite	8.0		9
Heptachlor, 2.5% granule on Attaclay	8.0		39
Aldrin, 5% granule on Attaclay	8.0		20
Aldrin, 5% granule on vermiculite	8.0		13
Dieldrin, emulsion spray	4.0		90
Dieldrin, emulsion spray	7.6		29
Endrin, emulsion spray	9.0		16
Thiodan, miscible spray	19.0		90
Check	—		143
L.S.D. 5% level			52

[a] Applied in a band 3 to 5 inches wide along seed furrow or over seedling plants.

Discussion Of Results.—As pointed out previously, in California, damage caused by the lesser cornstalk borer larvae is confined primarily to seedling plants. Damage arises for the most part from partly grown larvae resident within the field at planting time. As it is not possible to contact and kill the larvae with insecticides once they are established in a plant, successful control is predicated upon preventive applications. Consequently, insecticides are best applied at time of seeding up to the time when the seedling plants have just emerged from the soil. In comparing results obtained from applications applied at time of seeding with those applied to plants immediately after emergence, it appears that the latter time is slightly preferable to the former. As pointed out, however, in the experiment in which treatments were applied at time of planting, an irrigation immediately following may have rendered much of the insecticide ineffective. If the seed had been planted in moist soil so that irrigation would not be necessary until after the plants were established, better results might have been obtained.

Insecticidal control was effective only on plantings made on beds. Flat plantings for flood irrigation in which the seed was drilled into the soil in rows only a few inches apart resulted in poor control. It is reasoned that the same amount of toxicant per acre was much more concentrated on the more widely spaced bed plantings and, thus, more protection resulted. These results indicate that ground applications in which the insecticide is placed in a band no more than a few inches wide is the most effective method of application. Airplane or broadcast applications of any nature could not be expected to

be successful. On crops such as peanuts, which are subject to serious damage by this pest over a prolonged period of time, it may not be possible to obtain satisfactory plant protection with insecticides as used in these tests.

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Normal Cholinesterase Activity in the Erythrocytes of Cattle[1]

FLORENCE HERMENZE[2] and WILLIAM J. GOODWIN[3,4]

ABSTRACT

Fourteen groups of beef and dairy cattle belonging to Clemson College were employed in this experiment. A total of 75 animals were represented with 160 blood samples taken. At least two and usually three or four determinations were conducted on each sample for erythrocyte cholinesterase enzyme activity.

The electrometric method with the stated modifications was the basic method employed in determining the cholinesterase activity. (Michel 1949.)

It appears that variations in ambient temperature have no effect on bovine cholinesterase activity.

The mean for beef cattle was found to be delta pH/hr. 0.434 and for dairy cattle delta pH/hr. 0.410. Although the dairy cattle mean was slightly lower than the mean of beef cattle, it was not significant at the 5% level of probability.

The mean for the 14 groups of beef and dairy cattle was found to be delta pH/hr. 0.422 with its confidence limits of ± 0.074.

Several of the organic phosphate insecticides are showing promise for the control of external parasites of animals, therefore, a great many toxicological studies will probably be conducted in the near future. Since the inhibition of cholinesterase enzyme activity is one of the primary methods of determining the effects of these materials on various animals, it is necessary that a normal value of blood cholinesterase activity for each species be established.

Cattle were selected for this project since much of the insecticidal and toxicological work has been conducted on this species. All of the animals used in this experiment belonged to the Animal Husbandry and Dairy Departments of Clemson College. The cattle were purebred, healthy, well-fed and raised under ideal livestock conditions. Five bovine animals were employed in each group according to breed, sex, and age.

SEROLOGY.—Samples of 3.0 to 5.0 ml. of blood were obtained from the cattle by veni-puncture of the jugular vein. Blood was collected into 15.0-ml. graduated heparinized centrifuge tubes. The centrifuge tubes were prepared by evaporation of heparin solution calculated to allow 1.5 mg. heparin to 5.0 ml. blood. To prevent coagulation, the rubber-stoppered samples were gently agitated for uniform dispersion of the blood and heparin. The blood samples were placed in containers containing crushed ice for transportation to the laboratory for immediate separation of the erythrocytes from the plasma and subsequent cholinesterase determinations. A total of 75 beef and dairy animals were employed with 160 blood samples taken. At least two, and usually three or four, cholinesterase determinations were run on each blood sample.

METHOD OF ANALYSIS.—The electrometric method of Michel (1949) for the determination of cholinesterase activity was the basic technique employed in this study. The heparinized blood samples were centrifuged for 20 minutes at 2000 r.p.m. Plasma of bovine blood was removed by suction pump and discarded. Plasma was not used since previous experience had revealed no appreciable ChE activity by current test methods using either acetylcholine or butyrylcholine as substrates.

The erythrocytes were washed twice with 0.9% saline for 20 minutes in a centrifuge at 2000 r.p.m. After the second washing, the erythrocytes, diluted with an equal volume of saline, were used for the analysis. A 0.04-ml. sample of this cell suspension was measured into 1 ml. of 0.01% saponin. A special beaker for holding the blood aliquot throughout the procedure was used to facilitate handling. (Hamblin *et al.* 1953).

To the 0.04-ml. cell suspension in 1.0 ml. of saponin solution in the special vial, 1.0 ml. red cell buffer was added and the covered sample incubated 10 minutes in a constant temperature water bath at 25 degrees C.

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Table 1.—Normal erythrocyte cholinesterase activity of beef cattle.[a]

DESCRIPTION OF ANIMALS	AGE (MONTHS)	NO. OF ANIMALS	BLEEDING DATES (1955)	DELTA pH/HR. ERYTHROCYTES		
				Mean	S.D.	Range
(A) Hereford steers	12–13	5	2/ 8–2/17	0.421	0.068	0.330–0.493
(B) Hereford heifers	11–14	5	2/15–2/25–3/29	0.468	0.031	0.420–0.505
(C) Hereford heifers	5– 6	10	6/21–7/19 or 7/19–7/22	0.397	0.067	0.295–0.504
(D) Hereford heifers	12	5	3/29 —	0.479	0.089	0.390–0.575
(E) Hereford bulls	5– 6	5	6/21–7/22	0.484	0.041	0.428–0.527
(F) Hereford bulls	12–18	5	2/10–2/16	0.403	0.068	0.294–0.462
(G) Angus bulls	13–17	5	2/10–2/16	0.386	0.035	0.340–0.432
			Average	0.434	0.057	0.294–0.575

[a] 40 animals—80 blood samples.

(Michel 1949.) The initial pH of the sample was read using a Beckman Model G electrometer; 0.2 ml. (200 mg./10 cc.) acetylcholine chloride was added to the samples, the time was noted and the vial was replaced in the the 25 degree C. water bath for exactly 1 hour. At the end of exactly 1 hour the second pH reading was taken. The delta pH was the difference between the initial and final pH reading.

The original technique of Michel (1949) was modified to the extent that—A) 0.04 ml. of the packed red cell suspension in saline was measured directly into 1 ml. of 0.01% saponin as the blood aliquot and—B) the correction factor for non-enzymatic hydrolysis was established with each series of tests by a blank determination. This correction ranged from 0.01 to 0.06 delta pH per hour.

RESULTS AND DISCUSSION.—The seven groups of beef cattle are listed in table 1. Two breeds, Angus and Hereford, were used. A total of 40 animals were employed with 80 blood samples being taken. The mean for the seven groups of beef cattle was found to be delta pH/hr. 0.434.

Table 2 lists the seven groups of dairy cattle. Two breeds, Guernsey and Holstein-Friesian, were employed. A total of 35 animals were sampled and 80 blood samples were taken. The mean for the seven groups of dairy cattle was delta pH/hr. 0.410.

Although the mean for the dairy cattle is slightly lower than the mean for beef cattle, the difference is not significant at the 5% probability level.

The number of animals in each group, the age of the animals, the bleeding dates, the mean delta pH/hr., the standard deviation of the means, and the range of individual means are also included in tables 1 and 2.

The means for the 14 groups of cattle cannot be said to differ significantly from each other, and might well be represented by the grand average delta pH/hr. 0.422 with 95% confidence limits of ±0.074.

Table 3 gives the coefficient of variation with various choices as to the number of animals sampled, the number of times an animal was sampled, and the number of replicate determinations run on each sample. The results show variability within breeds and lead to the conclusion that

Table 2.—Normal erythrocyte cholinesterase activity of dairy cattle.[a]

DESCRIPTION OF ANIMALS	AGE (MONTHS)	NO. OF ANIMALS	BLEEDING DATES (1955)	DELTA pH/HR. ERYTHROCYTES		
				Mean	S.D.	Range
(H) Guernsey calves[b]	3	5	2/11–2/21	0.406	0.023	0.388–0.446
(I) Guernsey cows	2¾– 3 yr.	5	2/22–3/3–4/4	0.401	0.013	0.383–0.418
(J) Guernsey heifers	18 –20	5	6/17–6/28	0.395	0.014	0.385–0.420
(K) Holstein, Friesian calves[b]	3	5	2/11–2/21	0.393	0.045	0.352–0.470
(L) Holstein cows	2¾– 4½ yrs.	5	2/22–3/3–4/4	0.407	0.027	0.385–0.452
(M) Holstein heifers	18 –20	5	6/17–6/28	0.459	0.048	0.400–0.560
(N) Mixed heifers	14 –18	5	4/20–4/26	0.412	0.043	0.365–0.478
			Average	0.410	0.030	0.352–0.560

[a] 35 animals—80 blood samples.
[b] Female.

Table 3.—Normal erythrocyte cholinesterase activity of cattle.[a]

				HEREFORD					ANGUS	GUERNSEY			HOLSTEIN			MIXED
NO. OF ANIMALS	NO. OF SAMPLINGS	NO. OF REPLICATES	TOTAL NO. OF ANALYSES	Steers (A) %	Heifers (B and D) %	Heifers (C) %	Bulls (E) %	Bulls (F) %	BULLS (G) %	Calves, F. (H) %	Cows (I) %	Heifers (J) %	Friesian Calves, F. (K) %	Cows (L) %	Heifers (M) %	DAIRY HEIFERS (N) %
1	1	1	1	±36.6	±13.1	±19.0	±14.3	±39.6	±23.7	±9.0	±8.6	±12.3	±12.2	±8.3	±20.4	±11.1
2	1	1	2	±26.5	± 9.2	±13.5	±10.1	±27.9	±16.8	±6.4	±6.1	± 8.2	± 8.6	±5.9	±14.4	± 7.9
3	1	1	3	±21.2	± 7.5	±11.0	± 8.2	±22.9	±13.7	±5.2	±4.9	± 7.0	± 7.1	±4.8	±11.8	± 6.4
4	1	1	4	±18.3	± 6.5	± 9.5	± 7.1	±19.8	±11.9	±4.5	±4.3	± 6.2	± 6.1	±4.2	±10.2	± 5.6
5	1	1	5	±16.3	± 5.8	± 8.5	± 6.4	±17.7	±10.7	±4.0	±3.9	± 5.5	± 5.5	±3.7	± 9.1	± 5.0
5	1	2	10	±16.0	± 5.6	± 8.1	± 6.2	±16.8	± 9.4	±3.4	±3.6	± 4.7	± 5.3	±3.2	± 7.8	± 4.3
5	2	2	20	±15.6	± 4.6	± 7.9	± 4.4	±15.7	± 8.2	±2.5	±2.6	± —	± 4.9	±2.4	± 5.5	± 3.1

[a] Standard deviation of averages expressed as percentage for estimating the 95% confidence limits of a random cholinesterase determination.

Bovine ChE levels show greater variation between individuals than between breeds and that the variability is not due to the test itself.

Temperatures in South Carolina ranged from freezing to over 100°F. during the period of this study. To help evaluate the question of the effect of heat on ChE activity, blood samples were taken at 1 p.m. on one of the hottest days from cows whose erythrocytes ChE levels were known. There was no change in the individual erythrocyte ChE activity figures.

SUPPLEMENTARY NOTE.—Replicate tests have been performed on 134 blood samples using washed and unwashed erythrocytes. There was no significant difference in ChE activity by the T-test between washed and unwashed bovine red blood cells. Therefore it would save time to fill a capillary tube and centrifuge the venous blood, and measure the unwashed cells directly into the saponin solution.

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Evaluation of Insecticides for the Control of *Hippelates pusio* in Soil[1]

RICHARD P. DOW and MYRON J. WILLIS[2]

ABSTRACT

In plot tests evaluated by emergence traps, excellent control of *Hippelates pusio* was obtained with aldrin at 1 or 2 pounds per acre, chlordane at 4 pounds per acre, and BHC at 2½ pounds of gamma isomer per acre. The continued effectiveness of the original BHC application against a second generation of gnats was demonstrated by a second test begun 40 days after the first. DDT was found to be of little or no benefit at rates up to 16 pounds per acre.

When afternoon soil temperatures at a depth of 6 inches averaged 83 and 78 degrees F., the development of *H. pusio* from oviposition to the peak of adult emergence took an estimated period of 21 and 26 days, respectively.

In a test of emergence traps made where ants were abundant, the jars collected less than 25% of the adult gnats experimentally introduced. Another test indicated that relatively few gnats were removed from the collecting jars by scavengers. Means of improving the emergence trap are suggested.

Chloropid gnats of the *Hippelates pusio* group (*H. pusio* Loew, *H. collusor* (Townsend), and *H. bishoppi* Sabrosky) oviposit in freshly cultivated or otherwise disturbed soil and develop to maturity in that habitat. This finding has been established by the pioneer work of R. W. Burgess (Herms & Burgess 1930, Burgess 1951) and the definitive studies of J. T. Bigham (Bigham 1941, Dow *et al.* 1951). Because the larvae of these *Hippelates* are widespread in the soil, no insecticidal control seemed practical until the introduction of the chlorinated hydrocarbons made it possible to use chemicals which could be (a) applied in advance of their need and (b) expected to remain effective against more than one generation. The present paper summarizes the results of tests carried out in Brooks County, southwest Georgia, in 1951–52 to determine the effectiveness of various chlorinated hydrocarbons applied as soil insecticides for the control of *Hippelates pusio.*

TESTS OF BHC.—In Barwick, Georgia, an uncultivated field of very sandy soil with a heavy growth of hairy crabgrass, *Digitaria sanguinalis* (L.) Scop., was selected as an area to test BHC for the control of *Hippelates pusio.* First the grass was beaten down by dragging a heavy plank behind a jeep, and then two plots 120×180 ft. were laid out and staked. On August 17, 1951, after an emulsion of BHC was applied by constant pressure sprayer to one of these plots at the rate of 2½ pounds of gamma isomer per acre, the soil of both plots was disked. (A marginal strip of the untreated plot was first turned with a bottom plow which, because of the heavy grass, had to be put aside in favor of the disk harrow.) The immediate result of the disking was the attraction of large numbers of *H. pusio.*

Fifteen emergence traps placed at randomized locations on each plot on August 20 and 21 were put into operation by the addition of collecting jars on August 21. The inside dimensions of these rectangular traps (fig. 1) were 2×3 ft. The sides of the trap, 12 inches high, were of tongue and groove boards, nailed to external corner posts 2×2 inches in cross section. The top of the trap was a sheet of white canvas. Nailed to diagonally opposite corners on top of the trap were two blocks of wood bored to receive the top of the inverted 1-pint glass fruit jars which served as collecting chambers. In the mouth of each jar was placed an inwardly directed funnel made of cellulose acetate. This acted as a trapping device and also held dead gnats in the jar. The funnel had an opening 3/16-inch in diameter at the apex, and its base was glued to the rim of a jar lid from the center of which a disk 2⅛ inches in diameter had been removed. The funnel was held on the jar by the threaded ring (screw band) which

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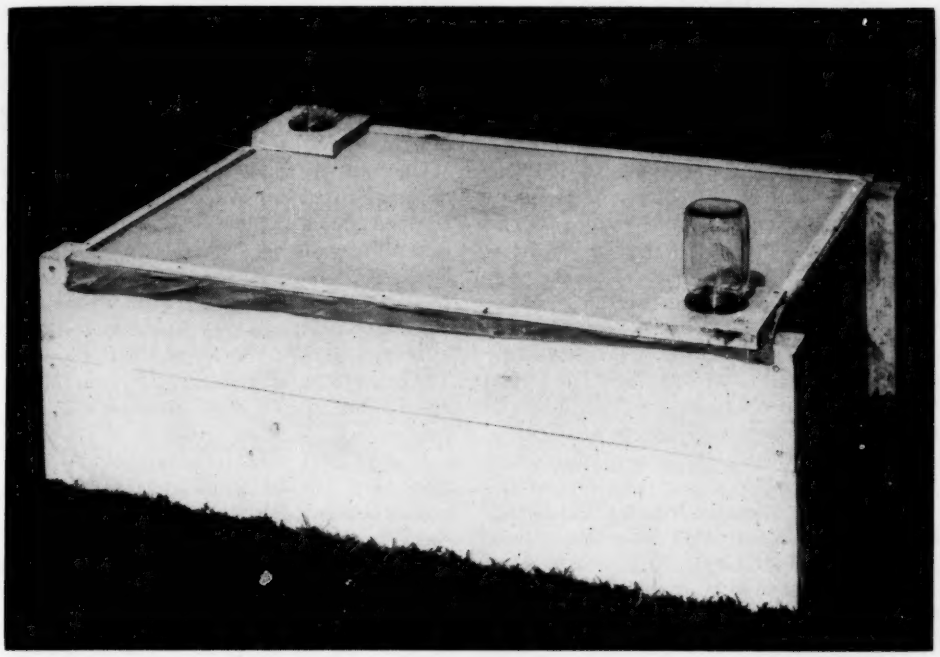

FIG. 1.—Emergence trap used to evaluate the emergence of *Hippelates pusio* from soil.

is made by the manufacturer to hold the lid. Under the wide range of weather conditions to which the emergence traps were exposed, the cone would frequently loosen from its supporting ring. The remedy found for this difficulty was to bind the base of the cone rigidly and snugly between two rings of sheet metal cut from jar lids.

Collections were made from the emergence traps at 3-day intervals. During the period when normal emergence was taking place on the untreated plot, only two *H. pusio* were trapped in the treated area (table 1). In the collections made on the untreated plot, there was great variation from trap to trap, some of which was associated with the amount of vegetation left on the surface of the ground. It is thought that where the vegetation was not turned under by the plowing and disking, the ground was less suitable for oviposition by *H. pusio*.

A second test of the same application of BHC was made after the collections of *H. pusio* from the untreated plot had dropped to a 3-day total of seven. The soil of both plots was disked on September 27, 41 days after the original treatment. After dark, on September 17, 28, and 29, five emergence traps were set on each plot at randomized locations and put into operation the same night. The first collection (of 1 to 3 days' duration) from all 30 traps was made on September 30. Thereafter collections were made at 3-day intervals as before. There was no apparent result of the different dates of trap setting, and there was no appreciable loss in the effectiveness of the BHC (table 1).

TESTS OF ALDRIN, CHLORDANE, AND DDT.—In 1952, a field of Norfolk sandy loam near Barwick, Georgia, which had never been treated with a chlorinated hydrocarbon, was selected for a 60-plot randomized block study of three insecticides. About an acre of this field, with a medium cover of weeds and grassy vegetation, was disked, plowed, fertilized with 1,000 pounds of 4-8-8, and redisked on June 12 and 13. Next, 60 plots, each 10×20 ft., were laid out lengthwise in 12 rows, 5 to a row. The interval between rows was 5 feet, and the interval between the plots within each row, 20 feet. The first plot of each of the 12 rows made up the first block of the test; the second plot of each row, the second block; etc. Each block included three check plots and a plot for each of three dosages of three insecticides. When the plots were sprayed

Table 1.—Control of *Hippelates pusio* by insecticidal treatment of soil.

DATES OF DISKING	PERIOD OF EVALUATION[a]	INSECTICIDE	TOXICANT PER ACRE (LB.)	*H. pusio* TRAPPED PER SQ. FT.
	Test 1, Treated August 17, 1951			
Aug. 17	13–40	BHC	2.5	0.02
		none	none	4.97
	Test 2, Treated August 17, 1951[b]			
Sept. 27	18–39	BHC	2.5	0.04
		none	none	2.24
	Test 3, Treated June 16, 1952			
June 12, 13, 16	13 & 14 to 39	aldrin	2	0.02
		aldrin	1	0.08
		aldrin	0.5	1.67
		chlordane	4	0.08
		chlordane	2	0.35
		chlordane	1	2.83
		DDT	16	1.44[c]
		DDT	8	3.45
		DDT	4	2.93
		none	none	2.99[d]

[a] Days after date of disking (first disking in Test 3).
[b] Original treatment of Test 1, retested by redisking same plots and repeating evaluation.
[c] Based on 8 traps instead of 10.
[d] Based on 30 traps.

on June 16, the application of the insecticides as emulsions was made by constant pressure sprayers inside a portable canvas fence placed around each area prior to treatment. By error, one dosage was omitted in one block. Immediately after the spraying, the whole field was disked lengthwise of the plots to a depth of at least 6 inches, and on June 17, the whole area was seeded with millet to provide green manure for replicate tests (never made) of the same applications. On June 25 and 26, two emergence traps were set at randomized positions 4 feet from each end and 2 feet from each side of all the plots except two in which the sprayed area covered less than the whole plot.

The first collection, made on June 30, was followed by six more at alternating 3- and 4-day intervals. The results (table 1) showed that aldrin at 1 or 2 pounds per acre and chlordane at 4 pounds per acre gave effective control of *H. pusio*, with chlordane at 2 pounds per acre providing a less satisfactory level of control. These results were confirmed by an analysis of variance supplemented by the procedure developed by Tukey (1949). The three most effective treatments were found significantly better than chlordane at 2 pounds per acre, and this, in turn, was shown to be significantly better than all the remaining treatments plus the controls. For the analysis of variance, the total number of *H. pusio* caught by each trap, plus one, was transformed to its common logarithm. This step effectively reduced the dependence of mean and variance which occurred in the within-plot variation calculated on a block basis from the raw data.

DISCUSSION.—In evaluating soil insecticides for the control of *H. pusio*, it is desirable to have the emergence traps in place a week before the peak of emergence. This requires some attention to the soil temperature. In the first test of BHC, the mean afternoon temperature 6 inches below the surface of bare soil in the untreated plot was 83° F. The peak of *H. pusio* emergence came in the collection period that was 19 to 22 days after the disking. In the second test of BHC, the mean soil temperature similarly measured was 78° F. and the peak of emergence occurred 24 to 27 days after the redisking. In the 60-plot study, the mean afternoon soil temperature at 6 inches was 95° F. The trap records did not show a definite peak of emergence: 261 *H. pusio* were taken in the first collection period, June 25 and 26 to June 30, and 267 *H. pusio* were taken in the second period, June 30 to July 3. Possibly the collection periods were untimely, but the fact that the last disking was 4 days after the first is believed to have prolonged the emergence period and thereby flattened its peak. In any case the peak emergence was probably not more than 18 days from the first disking. In the 60-plot study, temperatures taken at a depth of 6 inches underneath the emergence traps were found to average 5° F. cooler than those taken under exposed soil.

A remarkable feature of the ecology of *H. pusio* is its wide distribution over whole plowed fields. For example, in the 60-plot study, it was taken in both emergence traps on every untreated plot, and its occurrence elsewhere was apparently limited only by the applications of insecticides. In the collections made over untreated soil, however, the numbers of *H. pusio* taken per trap were subject to great variability, the principal cause of which is believed to be the activity of various predators and scavengers. The extent to which ants and similar organisms may affect the size of the trap catches is partly a question of what damage they would do to gnat populations under natural conditions and partly a question of how much they raid the collecting jars.

One test was made to learn if jars treated inside with a deposit of dieldrin would make possible, by killing the trapped gnats and preventing their escape at night, a longer interval between the times of collection. Eight emergence traps, four with treated and four with untreated jars, were set in the hard-packed soil of an uncultivated field, and approximately 100 gnats were blown into each of them on the first and third day of the test. The collections made on days 1 to 4 in the evening showed no significant difference between the treated and untreated jars, but the numbers of gnats recovered were only 23% of the first introduction and 22% of the total. Ants were abundant in the area used for this test and may possibly have been responsible for the low recovery of the introduced gnats.

In 1953, two insecticidal treatments for *H. pusio* in a pecan grove gave erratic results that were best explained by assuming that where the insecticide controlled the ants as well as the gnats, more gnats would be caught in the emergence traps. Accordingly, a test was made to find out if ants or other scavengers were removing gnats from the dieldrin-treated collecting jars which were then being used. The procedure was to collect gnats in a bait trap with a dieldrin-treated collecting jar, place 20 of these dead or moribund gnats in each of 30 fresh jars, and set these jars on 15 emergence traps, five of which were on untreated soil and five on each of the two treated areas. The jars were picked up on the fourth day. An actual loss of only 33 out of 600 gnats could be established, but even in this small total, the numbers that disappeared from the jars in each area were in line with the supposed depredations of ants in the preceding collection. It was therefore concluded that whether the gnats in the pecan grove were attacked before or after leaving the soil, any marked reduction of their numbers by ants probably took place before they reached the collecting jars.

If further tests indicate that, in an environment free of predators and scavengers, relatively few of the emerging gnats are trapped in the collecting jars, then it may be possible to make better use of the gnat's phototropism by substituting an opaque black cloth for the white canvas used to cover the emergence trap. If further tests indicate that predators and scavengers are making serious inroads on the specimens in the collecting jars, ant barriers should be devised. Though ants and similar organisms may always remain an unwelcome source of variability, their depredations should not be allowed to affect the actual trap catches. Making daily collections might prove to be necessary.

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The Employment of Exotic Entomophagous Insects in Pest Control[1]

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ABSTRACT

In order for an exotic entomophagous insect to establish itself when colonized in a new country, it must frequent the habitat of the host species at a time when the susceptible stages of the host are present. Establishment has maximum insurance when the colonizations are numerically adequate and are placed in contact with susceptible host populations which are not regressing. Effective entomophagous insects are usually derived from the native habitat of the host, provided that the host in that habitat exists at low population densities. The dominant entomophagous species obtained under such conditions is likely to be the most effective, particularly if it is host specific. Importations should be made from representative types of host environments, since no one species of entomophagous insect is likely to be effective throughout the range of its host. After establishment, artificial exposure of host populations provides a means of demonstrating the effectiveness of the entomophagous insect. However, where two or more entomophagous insects are concerned, evaluation may be difficult since the species which are responsible for the reduction of high host densities may not be the species responsible for the maintenance of the host populations at the average level or steady density.

Biological control programs for the protection of plants from injurious insects have, in certain instances, completely eliminated the need for chemical measures (Clausen 1956). The number of such instances may be increased by adherence to certain empirically derived principles (Thompson 1930, Williams 1931). The establishment of any exotic organism is usually, if not always, dependent upon a complicated set of circumstances (Smith *et al.* 1933).

During a 20-year period (1934–1954) in which some 80 species of biological races of parasitic Hymenoptera foreign to California were received by the writer and colonized under his direction, certain principles were exemplified. The importations were limited to species which can reproduce only by utilizing as food the body contents of pest organisms. Prior to colonization, each of these species was biologically analyzed under quarantine conditions to ascertain the nature of its host relations. Each was colonized under circumstances considered optimum for its reproduction. Twenty species[2] established themselves.

PREREQUISITES FOR ESTABLISHMENT.—Establishment is based upon certain more or less obvious prerequisites. An entomophagous insect can establish itself only if it frequents the types of environments frequented by its host. Contact between the entomophagous species and the host species is in most instances brought about by the gravid females of each species frequenting either synchronously or asynchronously the same environment.

The original releases of the imported material are made under favorable meteorological conditions and are so timed that the entomophagous insect can contact the host when the latter is in a stage susceptible to attack. The new host habitat must function, temporarily at least, as a "trap" for the gravid female of the entomophagous species if the species is to reproduce for more than one generation and to become established (Flanders 1940a). The importation of natural enemies ordinarily commences from regions similar ecologically to the region in which they are to be colonized, the latter region then being likely to contain favorable habitats.

The food supply needed by the adult may delimit the areas in which an entomophagous species can establish itself. The adult of *Tiphia vernalis* Roh., for example, requires food which consists almost exclusively of the honeydew of aphids, so that colonizations in areas where such food is lacking are abortive (Clausen *et al.* 1932).

Establishment may be facilitated if the colonized material is field-grown or at least allowed to emerge as adults in the field. The red-scale-feeding form of *Prospaltella perniciosi* Tower is recovered most readily when the releases consist of full-fed larvae or pupae within their mummified hosts, a result of the fact that under mass-culture conditions the adult, when collected for release, has already deposited most, if not all, of its full complement of eggs. The establishment of *Scolia ruficornis* Burm. in Micronesia was facilitated because it was released while in its cocooned stage, the cocoons being "planted" in heaps of compost which were infested with the larvae of the host, *Oryctes rhinoceros* (L.). The *Scolia* adults emerging from the cocoons tended to remain near the compost heap, although they foraged for flower nectar some distance away. Mating and oviposition were thus facilitated (C. E. Pemberton, *in litt.*).

Colonizations are made preferably on either a static or an expanding host population. Releases made on a regressing host population are at a great disadvantage since they are then subjected to host-depressing factors such as

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[2] *Anarhopus sydneyensis* Timb., *Allotropa citri* Gahan, *Aphelinus mali* (Hald.), *Aphytis lingnanensis* Comp., *A. lepidosaphes* Comp., *Coccophagus cowperi* Gir., *C. insidiator* (Dalm.), *C. pulvinariae* Comp., *C. rusti* Comp., *Comperiella bifasciata* How. (red scale race), *Habrolepis rouxi* Comp., *Lecaniobius utilis* Comp., *Metaphycus helvolus* Comp., *M. stanleyi* Comp., *Pauridia peregrina* Timb., *Physcus flaviceps* Gir., *Prospaltella elongata* Doz., *P. perniciosi* Tower (two races, eastern U. S. and East Asia), *Tetracnemus peregrinus* Comp., and *Tetrastichus brevistigma* Gahan.

other natural enemies and lack of food. The developmental mortality of endoparasites may be least in host populations which are healthy and vigorous, as the writer has observed with the hymenopterous parasites *Diversinervus smithi* Comp. and *Coccophagus rusti* Comp.

The density of the pest population upon which colonizations are made must be such that the natural enemies can increase and disperse before the pest population is destroyed either by other natural enemies, by overcrowding, by the development of host-plant phaenoimmunity, or by the killing of the host plant.

Since the adaptiveness of a species released in a new environment is likely to be a function of environmental selection or "screening" of the first generation, large numbers should be colonized in proper sequence on host populations in condition for attack.

The establishment of a biparental species, when only small numbers are available for release, may be facilitated by briefly confining the released individuals to a small segment of the field population of the host within a small cloth cage. This procedure tends to insure the occurrence of mating in the following generation.

When establishment is attempted with only a small amount of material, the colonies should be released in localities where the numbers of indigenous general predators such as spiders and ants are at a minimum. After a species of natural enemy is established, such predators have little, if any, direct influence on its general effectiveness.

The natural enemy of a phytophagous insect, if not known to be adapted to monocultural conditions, should be colonized first in a plant community characterized by a variety of host plants, for in such a community the susceptible stages of the host insect are likely to occur in a sequence most favorable for the establishment of the natural enemy. In a pest-infested monoculture a lack of uniformity in plant development tends to increase the controlling action of natural enemies.

Evidence of effective control of an organism in its country of origin by natural enemies is of practical value in determining the area to be explored, the time spent in searching, and the priority and sequence of collecting and shipping the several natural enemies.

Entomophagous insects vary greatly in their capacities for reproduction at low host densities. Consequently the importation of effective natural enemies is facilitated by collecting procedures tending to select those which most readily reproduce at noneconomic densities of the host.

PREREQUISITES FOR OBTAINING EFFECTIVE SPECIES. The chance of importing and establishing effective natural enemies is enhanced if the circumstances of the original collection and the conditions of colonization in the destination country (Flanders 1940c) are arranged according to one or more of the following principles and procedures.

A. The native habitat of an organism is the most likely source of its effective natural enemies.

This principle was recognized in 1854 by Asa Fitch when he speculated that the chief, if not the sole, cause of the development of the wheat midge, *Sitodiplosis mosellana* (Gehin), as a pest in North America was the lack in that region of "nature's appointed means" of repression, namely, parasites, which in Europe usually kept the midge under economic control (Howard & Fiske 1911).

In general, the relation between a host species and its effective parasite species is mutually beneficial. Since the benefit to the host species is derived from having a population level less than the limit set by space and food supply, it is reasonable to assume that the older the host-parasite relation, the greater the evolutionary development toward minimum population levels. Such a development may be brought about by an increase in the searching capacity of the parasite.

When the scarcity of a pest species is the result of the action of an entomophagous insect, the interacting populations tend to occur in more or less discrete communities (episodes) that wax, wane, and disappear (Nicholson & Bailey 1935). Such a spotted distribution is accentuated in any insect species which tends to occur in colonies independently of the host-parasite relation. At the low densities of such species there is little, if any, overlapping in the diffusion of its progeny from the parental locations (Smith 1939). The occurrence of highly localized episodes in the field under conditions which generally are nutritionally and physically favorable for the reproduction of the pest is presumptive evidence that an effective natural enemy is operating.

An outstanding ecosystem of natural enemy-caused episodes results from the control exerted by the coccinellid *Rodolia cardinalis* (Muls.) over the cottony-cushion scale, *Icerya purchasi* Mask., in California citrus orchards. A similar situation with respect to the natural control of aphids on citrus was observed by the writer in south China (fig. 1).

During the first 6 months of 1954 the new foliage on all varieties of citrus appeared free of aphids except for rarely occurring very small infestations. These infestations were limited to twigs less than 2 feet in length. The injurious effect of these aphid populations was of no importance, each episode being short-lived (in spite of attendance by ants) because of the action of several species of natural enemies.

As pointed out by Elton (1946), phytophagous animals do not normally become limited in numbers by starvation. The continuous existence of one or more species on a common and plentiful food supply is based on the fact that the densities of their populations govern the controlling action of their natural enemies (Nicholson & Bailey 1935). It is interesting to note in this connection that Elton considered the ability of certain groups of unrelated species to exist together in the same area while drawing upon the same pool of resources to be one of the unsolved problems in population dynamics.

B. The scarcity of the pest in the area to be searched is a primary requirement for the collection of the effective natural enemies.

Only when a host population in its original habitat is maintained at a relatively low density by natural enemies can the attainment of such a balance in the destination country be expected.

It is apparent that the natural enemies obtained from areas where the host generally occurs at low densities are likely to be most effective, provided, of course, that the nutritional and physical factors in such areas are favorable for the reproduction of the host (Smith 1933).

In obtaining material for shipment to the country of

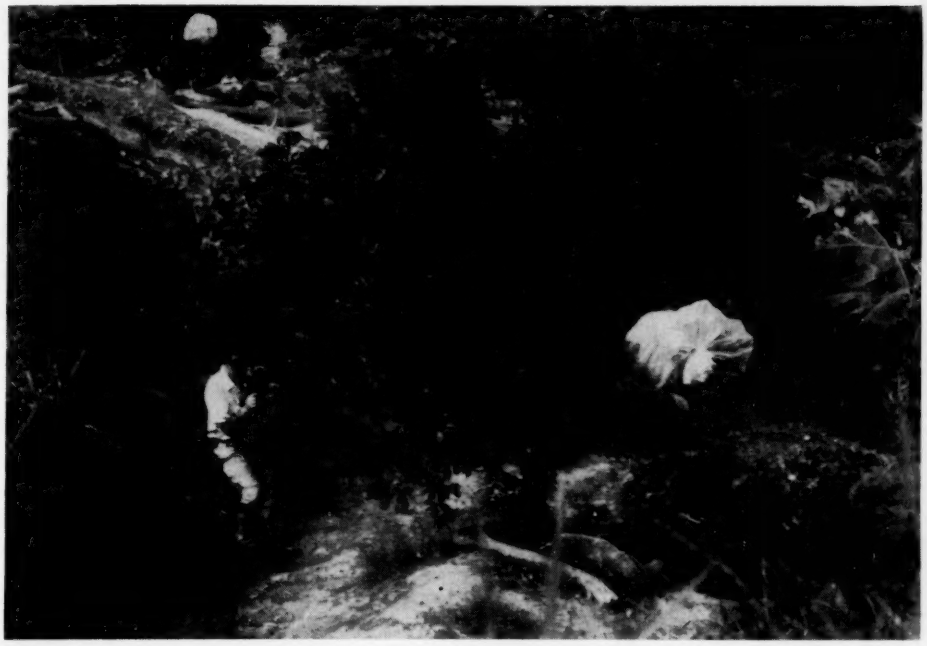

FIG. 1.—Grapefruit trees in south China, showing branches encased in organdie bags to allow increase of aphid populations free of natural enemies. A heavy aphid infestation exposed by removal of the bag completely disappeared within a week as a result of the action of natural enemies.

destination, three procedures may be utilized:

1. The collection of parasitized individuals.
2. The field exposure of unparasitized hosts utilizing either artificial cultures or protected field populations.
3. The collection of free-living natural enemies.

The first procedure is time-consuming but requires a minimum amount of equipment. The collection of only a few specimens may be all that is necessary if air-transport is quickly available and reproduction facilities in the country of destination are adequate.

The effective natural enemy in regions of host scarcity may be most readily collected by means of the "host-exposure" method in which infestations of the host organism are artificially produced and then exposed to attack in the field. C. E. Pemberton (*in litt.*) informed the writer that in 1930 he used this method to good advantage in searching for egg parasites of the grasshopper *Oxya chinensis* (Thun.).

On the Malay Peninsula he found *Oxya chinesis* to be extremely uncommon in the grassy areas where it occurred naturally. After digging for weeks with a pickax and failing to unearth any egg masses, he built a cage 36 inches square, with the sides and top covered with coarse screen and the bottom open. The mesh of the screen was of a size sufficient to confine the females of *O. chinensis* but to permit the entrance of parasites. This cage was placed in a grassy field of about 500 acres.

By sweeping the grass over the countryside he was able to obtain in one day 25 to 30 grasshoppers. When these were placed in the cage the females promptly oviposited in the soil. After 2 weeks' exposure to parasitization, the egg masses were dug up and placed in vials. A few weeks later nearly every egg mass yielded quantities of the egg parasite *Scelio pembertoni* Timb. This parasite, upon its introduction and establishment in Hawaii, effected a remarkable control of *Oxya chinensis*.

As stated by Pemberton (*in litt.*): "No parasite hunter should neglect to use the exposure method when the ecology of the pest lends itself to its use." He added, whimsically, "I suspect that I would still be digging for grasshopper eggs in Malaya if I had not resorted to this simple method."

The writer has successfully used the "host-exposure" method in obtaining natural enemies of citrus aphids in south China and parasites of *Saissetia* spp. in Brazil. If no natural enemies can be collected by the "host-exposure" method, the scarcity of the host may be attributed to an inadequate food supply or some other limiting requisite (Nicholson 1957).

The collection of free-living natural enemies[3] is attempted only when the environments in the particular locality searched are known to be of the type frequented by the pest insect whose control is desired in the country of destination. The collections are usually limited to the parasites and predators belonging to families that include species known to attack the pest insect or a species related to it.

C. The dominant species of natural enemy obtained at the minimum density of the pest population is likely to be the most effective species in a new habitat unless the dominance in the original habitat is an effect of reproduction on other hosts.

D. The natural enemies of a phytophagous pest should be collected, if possible, from the species (or variety) of host plant on which in the country of destination they are to be established.

[3] When collections are made by the beating of plant foliage, a satisfactory "beating" receptacle or pad consists of a white cloth tautly stretched across a metal ring and having an area of fine nap which can be quickly scanned. This pad, when placed properly beneath a small branch of a tree, receives such insects as are dislodged from the branch by several sharp blows with hand or stick.

This rule is based on two phenomena: (a) the attractiveness of the host plant to the natural enemy as a habitat may be the first step in the finding of the host insect, this phytotropism of an entomophagous insect being well-illustrated in the mirid *Cyrtorhinus mundulus* Bredd, the most effective agent in the control of the sugarcane leafhopper, *Perkinsiella saccharicida* Kirk., in Hawaii; and (b) the host insect may consist of two or more races characterized by different host plants and different parasites, as with the polyphagous mealybug *Pseudococcus maritimus* (Ehrh.), which includes a race that infests only the grape, this race having a parasite, *Acerophagus notativentris* (Gir.), peculiar to it (A. J. Basinger, unpublished notes).

None of the numerous parasites of the black scale, *Saissetia oleae* (Bern.), introduced during the past 50 years into California from their native habitat in Africa, has given complete and general control of the black scale on citrus, a plant native to Asia. In Africa, black scale is rarely found on citrus (Compere 1940), an effect of either the unsuitability of citrus in that region for the reproduction of the scale or the presence there of citrus-inhabiting, effective natural enemies. Since none of the parasites sent to California was obtained from citrus, it is possible that the lack of control results from the parasites not frequenting that plant. If such is the case, artificial infestations of black scale on citrus in Africa might turn up more effective species of parasite.

It is noteworthy that the parasite *Metaphycus helvolus* (Comp.), introduced into California from Africa in 1937, gives sporadic control of *Saissetia oleae* on citrus but complete control of *S. nigra* (Niet.) on such plants as *Pittosporum undulatum* and *Rhus integrifolia*.

California red scale, *Aonidiella aurantii* (Mask.), when infesting agave, avocado and rose, appears to be immune to attack by certain of its natural parasites, apparently because these plants are not visited by the adults of such parasites. However, when this scale inhabits citrus it is a host for more different kinds of parasites than are known to attack it on any other plant (Flanders & Gressitt 1958).

E. Importation work against a pest cannot be considered completed until all geographical races of natural enemies have been discovered, collected, and released against it.

Geographical races may differ in their capacity for controlling the same host species (Clausen 1936, Smith 1941, Flanders 1950, 1957). The colonization of more than one species of natural enemy may not be needed to obtain, within a limited area, satisfactory control of a pest. However, a single natural enemy is rarely, if ever, able to occupy all the habitats of its host effectively, so that the maximum general control of a pest requires the importation and colonization of more than one species. The more kinds of natural enemies established, the more effective is such control (Smith 1929).

The total action of several natural enemies of a pest is not as likely to be adversely affected by environmental changes as is the action of only one species (Smith 1929).

A natural enemy may give satisfactory control of a phytophagous insect within a community of several host plants but fail to do so over a large area in which there is only one kind of host plant (Schneider 1939).

F. A specific natural enemy should have priority in colonization as well as in shipment, if that enemy is found in competition with one or more supposedly effective nonspecific natural enemies.

The action of effective nonspecific natural enemies may preclude effective control action by a specific natural enemy. In such a case an effective specific natural enemy may be extremely scarce and consequently underrated.

If one or more nonspecific natural enemies are responsible for maintaining the host of a specific natural enemy in its native habitat at low densities, then the specific form acting alone in a new habitat is likely to be equally effective. Also, since the searching of a specific natural enemy is independent of the presence of alternate hosts, its controlling action is likely to be more dependable.

The introduction and establishment of a specific natural enemy is likely to occur more readily than the introduction and establishment of a complex of nonspecific natural enemies, particularly when the latter require alternate hosts for reproduction.

OBTAINING PROOF OF THE EFFECTIVENESS OF AN ENTOMOPHAGOUS INSECT.—Should the establishment of an exotic entomophagous insect be followed by a scarcity of the host species, the most reliable means for experimentally verifying the action of that insect as the cause of the scarcity involves the use of some form of the "host-exposure" method.

The adults and immature instars of the entomophagous species may be artificially removed from a small segment of the host-habitat as soon as they appear so that the host population increases naturally or the host may be cultured in the laboratory in numbers sufficient to create under natural conditions an infestation properly distributed in time and in space. Such infestations must be maintained under observation so constant that the mortality factors that eliminate them can be ascertained.

The destruction of a heavy infestation by natural enemies provides no evidence as to the identity of the enemy or enemies responsible for maintaining the host population generally at low densities. In California heavy infestations of *Coccus hesperidum* L. are usually destroyed either by *Metaphycus stanleyi* Comp. or by *Coccophagus scutellaris* (Dalm.), not by *Metaphycus luteolus* (Timb.), the most effective species at low host densities. The reproductivity of *M. luteolus*, unlike that of *M. stanleyi* and *C. scutellaris*, is inhibited by ants attending its host (Timberlake 1913).

With plant-feeding homopterous insects that occur naturally in maximum numbers only in the presence of ants, the subsequent elimination of such infestations by the action of a natural enemy (Flanders 1945) is not in itself proof that the general scarcity of the host is also the work of this particular natural enemy. Smith (1937) stated that such proof is "almost" conclusive. The inconclusive nature of such proof results from uncertainty as to the means by which ant-protected field infestations are initiated (none has been initiated experimentally) and the extent to which the increase of such infestations is a direct effect of ant-attendance (Clausen 1956). It is possible that ant-promoted populations, when freed of ants, are particularly susceptible to attack by natural enemies, non-ant-promoted populations being relatively immune (Smith & Armitage 1931).

A natural enemy which destroys an ant-protected host population is likely to be a nonspecific species unable

to exist in effective numbers on low densities of the species thus destroyed. It is noteworthy that most, if not all, of the ineffective parasites of the black scale, *Saissetia oleae*, collected in Africa and released in California were apparently obtained from infestations protected by ants or isolated otherwise from effective natural enemies.

Field studies concerning the relative importance of the various natural enemies of a pest can be quite contradictory. During the years 1943 to 1946, inclusive, unusually high densities of the longtailed mealybug, *Pseudococcus aonidum* L., on citrus in southern California were the occasion of an intensive study of the natural enemies involved (DeBach 1949). This study showed that at high host densities the effect of the imported parasite *Anarhopus sydneyensis* Timb. in controlling the populations of the mealybug was markedly limited by competition from predators and by the parasitic action of *Lygocerus* sp., a general hyperparasite of mealybugs. The conclusion was reached that the predators would be more effective than the parasite *Anarhopus* even if the latter were not subject to these limiting factors. Significantly it was the ineffectiveness of these predators which 10 years earlier had led to the importation of *A. sydneyensis*. Other studies have repeatedly shown parasitization of mealybug populations by *Anarhopus* on citrus and avocado to be economically effective (Flanders 1940b, 1944, C. A. Fleschner, unpublished notes).

The effectiveness of a newly colonized natural enemy is observable during its year-to-year dispersal from the points of release if, during that dispersal, it destroys in succession economic populations of its host. Such effectiveness is well demonstrated when introductions into different regions are made in sequence and there follows in each region an economic reduction of the host populations (Smith 1939).

CONCLUSIONS.—The native habitat of a pest is the most likely source of an effective natural enemy. The sources of an exotic entomophagous insect should include, if possible, the kind of plant or animal upon which the host insect in the country of destination is a pest. The entomophagous insect which is the dominant natural enemy at a low density of a pest species is most likely to prove effective, provided that other host species at the time of collection were either rare or lacking. A potentially effective natural enemy may not be dominant at any density if it is specific in its host relations and exists in direct competition with effective nonspecific natural enemies.

Biological control projects hitherto unsuccessful, which were not operated on these bases, may be reactivated with profit. In such projects the colonized natural enemies may not have represented all the types of environments in which the pests occurred at low densities nor have included any derived from the kind of pest-infested organism, plant or animal, upon which they were colonized.

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Determination of Bayer 19639 Residues in Agricultural Crops by Cholinesterase Inhibition[1]

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Abstract

Bayer 19639 (Di-syston), a very weak cholinesterase inhibitor, is oxidized to a much stronger inhibitor by a mixture of hydrogen peroxide and acetic acid. Using this activating procedure, Bayer 19639 residues are detected in agricultural crops by a cholinesterase inhibition method.

Bayer 19639 [O,*O*-diethyl *S*-2-(ethylthio)ethyl phosphorodithioate], a development of Farbenfabriken Bayer is an insecticide manufactured under license in the United States by the Chemagro Corporation under the trade name Di-syston. Its experimental use on agricultural crops led to a search for a method of analysis in order to determine residues on these crops.

The method developed at this laboratory is one that utilizes a modification of the Giang and Hall cholinesterase inhibition determination (Giang & Hall 1951) using an oxidation with peracetic acid to increase sensitivity.

Original attempts to analyze for Bayer 19639 by the cholinesterase inhibition method were not successful since this compound is not a strong inhibitor in its pure state (Metcalf *et al.* 1957). Attempts to increase cholinesterase inhibition activity by oxidation with several oxidizing agents were partially successful in that more sensitive standard curves could be prepared. However, addition of plant extractives produced erratic recoveries of added Bayer 19639.

The oxidizing agents tried were bromine water (Fallscheer & Cook 1956), bromine in chloroform and N-bromosuccinimide. These materials lowered the amount required for 50% cholinesterase inhibition from 110 micrograms to 7 to 8 micrograms. The volumes and methods used are practically the same as those described later.

The method described below is the one which gave successful results. It is very similar to the method used for the analysis of Trithion (Lubezoff *et al.* 1958).

Apparatus:

1. *pH meter*, Beckman Model G, or equivalent.
2. *Constant temperature bath*, maintained at 75° ± 1° C. equipped with a rack that keeps the test tubes in a vertical position immersed to 4–5 cm.
3. *An incubator*, maintained at 37.5° ± 0.5C°. A water bath of the same temperature, and equipped with the proper rack for holding the vials and beakers, can also be used.
4. *Solvent evaporator*, a hair drier used in conjunction with a sand bath maintained at not over 50° C.
5. *5 ml. shell vials*, or micro beakers, similar to those supplied with the pH meter. A set of 24 was used in the present work. (The number mentioned for the following is that necessary to run such a set of 24 measurements of pH.)
6. *Glass stoppered test tubes*, 160×16 mm. A set of 12.

Reagents:

1. *Peroxide—acetic acid solution*, one part by volume of 30% hydrogen peroxide is mixed with 5 parts by volume of glacial acetic acid. This mixture is prepared a few minutes before it is used. Any quantities not used are discarded.
2. *Benzene*, technical grade
3. *Bayer 19639 stock solutions*, a solution of Bayer 19639 in benzene containing 10 micrograms/ml. was used as a standard. Solutions containing 100 micrograms/ml. and 1000 micrograms/ml. were also prepared. All solutions were kept in refrigerator when not in use.
4. *White oil solution*, used to soften residues. A 2% solution of white (refined) mineral oil in benzene.
5. *Human blood plasma*, as a source of cholinesterase. It is kept refrigerated. In one case, outdated plasma purchased from the local blood bank and kept refrigerated in the original plastic bag was satisfactory for 2 to 3 months from the date of purchase. Normal precautions were taken to avoid contamination.
6. *Buffer solution* of a pH of about 8.

Sodium barbital	7.42 gm.
Potassium chloride	89.5 gm.
Potassium dihydrogen phosphate	1.089 gm.
Distilled water	to 1 liter
Toluene (as a preservative)	1 drop

Solution kept in refrigerator when not in use.

7. *Acetylcholine solution* 0.66 M

Acetylcholine bromide	14.924 gm.
Distilled water	to 100 ml.
Toluene (as a preservative)	1 drop

Solution kept under refrigeration at all times, when not in use.

Procedure.—The analytical procedure consisted of the following steps: (a) Bayer 19639 oxidation, (b) cholinesterase inhibition, (c) preparation of standard curve (d) determination of Bayer 19639 in the sample.

A. *Bayer 19639 Oxidation*:

1. Transfer aliquot of solution containing from 5 to 20 micrograms of Bayer 19639 into a 20-ml. beaker. Evaporate solvent on evaporator to just dryness. Take up in (add) 5 ml. of benzene. Stir lightly. Transfer to glass stoppered test tube.
2. Add 3 ml. of peroxode-acetic acid solution and a tiny piece of boiling chip to the tube.
3. Stopper tube, shake briefly, loosen stopper, and place in 75° C. bath for 20 minutes.
4. Remove test tube from 75° C. bath and cool in ice bath for a few minutes.

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5. Add to tube 5 ml. distilled water, stopper, and shake well.
6. Add 4 drops of white oil solution to each 20 ml. beaker.
7. When benzene layer (top layer in tube) has cleared, transfer measured aliquots into the 20-ml. beakers. These aliquots should contain between 0.6 and 2.0 micrograms of Bayer 19639 in order to obtain an inhibition of cholinesterase within the reliably measurable range.

   The data presented in this paper are based, for each particular sample, on at least three different aliquots within the favorable range.
8. Remove benzene with solvent evaporator. Allow a few minutes after beakers appear dry to remove any trace of acetic acid. (This can be detected by odor).
9. Add 5 ml. distilled water to each beaker. Cover each beaker with aluminum foil. Place beakers for 10 minutes on a hot plate maintained at 120° C., using caution to prevent volume loss.

   At this point the beakers are usually stored overnight, and the cholinesterase inhibition is carried out on the next day.

   Figure 1 shows effect of time of oxidation upon 50% inhibition values for Bayer 19639.

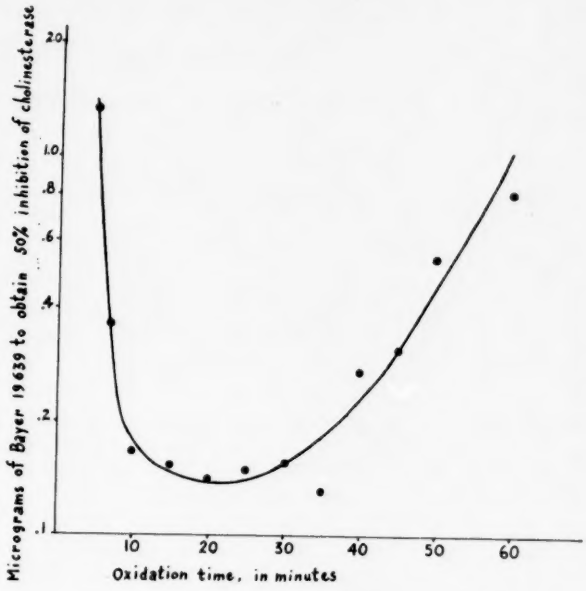

FIG. 1.—Effect of time of oxidation upon 50% inhibition values for Bayer 19639.

B. *Cholinesterase Inhibition:*

1. Add 0.6 ml. of plasma to each beaker through the aluminum foil cover of the beaker[3] stirring thoroughly. A hypodermic syringe was used in our case.
2. Place beakers in 37.5° C. incubator for 70 minutes.
3. Remove covers and transfer a 1-ml. aliquot from each beaker to a 5-ml. shell vial to which 1 ml. of buffer solution has been added.
4. Take pH of each vial. This is recorded as the initial pH.
5. Add carefully to each vial 5 drops of acetylcholine solution (0.2 ml.).
6. Place beakers in 37.5° C. incubator for 2 hours.
7. Allow to cool for about 5 minutes, and take the pH of each vial. This is recorded as the final pH.
8. Calculate the per cent of cholinesterase inhibition as follows

$$\% \text{ ChE inhibition} = \frac{\text{sample } \Delta\text{pH} \times 100}{\text{Blank } \Delta\text{pH}}$$

ΔpH is the difference between initial and final pH.

The blank is a sample of distilled water (containing no Bayer 19639 or plant extractives) which has been processed (starting at step 9 of oxidation procedure) in the same way as the samples of the same set. Distilled water blanks are used so that contaminated check samples can be detected. The ΔpH of the blank should range from 1.8 to 2.0 pH units.

Blanks were run as the first two and the last two samples of a 24-sample run. As the hydrolysis of acetylcholine continues during the time the final pH is taken, the last blanks will usually have a pH of about 0.15–0.20 units lower than the first. For best accuracy, this must be taken into account when using the above formula, to calculate per cent inhibition.

C. *Preparation of Standard Curve:*

1. Transfer 5 ml. of a stock solution containing 10 micrograms Bayer 19639 per ml. to the special tube. Oxidize in the same way described for samples (steps 2 to 9). In step 7 take aliquots of 0.05, 0.06, 0.08, 0.10, 0.15, 0.20, 0.25 ml. in duplicate.
2. Proceed to the cholinesterase inhibition, including the necessary blanks (usually 2 or 4) and calculate the per cent of inhibition.
3. On semi-log graph paper, plot per cent inhibition against dosage (in micrograms) of Bayer 19639. (Dosage on the log scale.) The standard curve for Bayer 19639 thus obtained, is shown in figure 2. The part of the curve between 20% and 75% inhibition is approximately a straight line, and is used as a standard for comparison with the inhibition caused by each sample.

When the same reagents and plasma are used, the slope of this line does not change from one run to another. Its position, however, usually changes. For the sake of accuracy, an aliquot of the Bayer 19639 stock solution, usually containing 1 microgram (0.10 ml. of the stock solution), is included in duplicate in each run of samples. The inhibition thus obtained, when plotted against the dosage, will show the point(s) through which the standard curve should pass in that particular run of samples. (See fig. 2.) A parallel to the standard curve is then drawn through this point and is used as the standard curve for comparison at that particular run (day).

When new batches of plasma or other reagents are used, or the daily standards change considerably, the curve should be reestablished.

[3] Different units of plasma differ in their ChE content and therefore, the proper amount to be added to each beaker may differ from 0.6 ml. The amount that we used was determined by trying different amounts, and selecting the one which gave a ΔpH of 1.8–2.0.

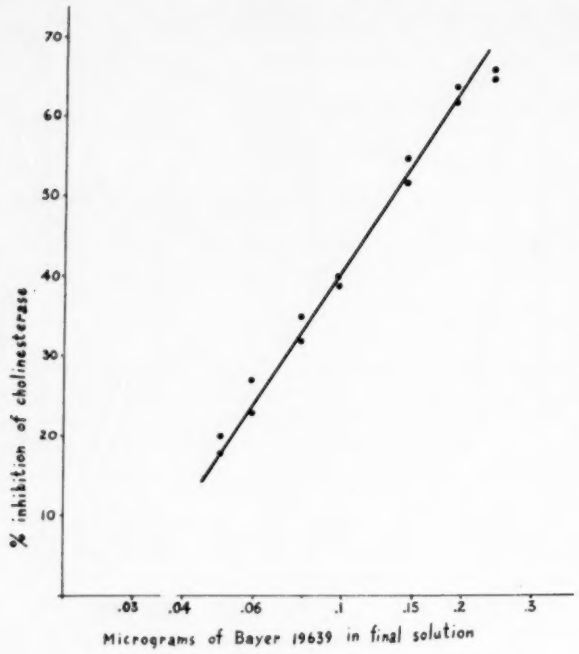

FIG. 2.—Standard curve for oxidized Bayer 19639 (Oxidation time 20 minutes).

D. *Determination of Bayer 19639 in Sample Extracts:*

Plant samples are thoroughly chopped and are extracted by tumbling for 1 hour in the presence of chloroform. The chloroform extract is dehydrated by passage through anhydrous sodium sulfate. An aliquot of the sample estimated to contain from 5 to 20 micrograms is taken and the procedure carried out as described previously. The size of this aliquot is a matter of experience and knowledge of the history of the crop from application of the chemical to extraction of sample. If such data are not available or there has been no previous experience with a particular crop, it will be necessary to try a wider range of aliquot amounts or even to repeat the procedure within the favorable range. The Bayer 19639 content of a sample is calculated by comparison with the standard calibration curve. (See fig. 2.)

Table 1 illustrates recoveries of Bayer 19639 added to several plant extractives.

Table 1.—Recovery of Bayer 19639[a] for fortified control sample extracts.

Crop	Sample Group	Grams of Crop Analyzed	Micrograms Bayer 19639 per Gram of Crop		Per Cent Recovery (Average)
			Added	Found (Average)	
Potato tuber	I	5	0	0.76	—
	I	5	1.0	1.50	74
	I	5	2.0	2.54	89
	I	5	10.0	11.20	104
	II	5	0	1.28	—
	II	5	1.0	2.13	85
	III	2.5	0	0	—
	III	2.5	2.0	1.80	90
	III	2.5	10.0	6.20	62
Potato foliage		5	0	11.70[b]	—
		5	10.0	22.80	111
Cabbage		5	0	0	—
		5	1.0	0.84	84
		5	0.5	0.25[c]	50
Broccoli	I	5	0	0	—
	I	5	1.0	0.86	86
	II	5	0	0	—
	II	5	1.0	0.93	93
Soy beans		2.5	0	0	—
		2.5	2.0	1.36	68
		2.5	4.0	2.84	71

[a] Added the day of analysis.
[b] A sample from a sprayed plot (not a control).
[c] Bayer 19639 added to this sample 307 days before analysis. Extract kept in refrigerator.

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Factors Affecting the Biology of Cattle Lice[1]

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Abstract

Populations of the biting cattle louse, *Bovicola bovis* (L.), were intensively studied on 12 heifers representing four dairy breeds. Results of these studies demonstrated that this species was cyclic in occurrence reaching a peak of infestation during late January or early February, and that Holstein cattle were more heavily infested than either Guernsey, Jersey or Brown Swiss breeds. In an attempt to find factors affecting intensity of infestation and cyclic occurrence it was found that these phenomena were not associated with individual hair diameter or length, with density of hair per unit area of skin surface, or with ether-alcohol soluble skin secretions. A positive correlation, however, was found between hair thickness and population level within the Holstein breed. It was also demonstrated that the amount of skin scurf was greater during the summer months than during the winter months of high population levels.

Every winter lice present a serious problem on cattle in the northern portion of the United States. Although much is known of the life history and control of lice, little information is available concerning their ecology. It is commonly known that louse populations are greatest during winter months and apparently absent during the summer. Clipping appears to be an effective means of control for chewing lice. Re-establishment of heavy infestations on animals clipped early in the season, however, may occur and these infestations may be prolonged beyond the normal seasonal incidence (Allen & Dicke 1952). Cyclic occurrence of lice has been attributed to crowding, diet, light intensity, and temperature but the evidence is not conclusive (Lamson 1918, Imes 1940, Craufurd-Benson 1941).

Variations in susceptibility have also been reported among the dairy breeds. Holsteins have been generally considered particularly subject to louse infestations (Shull 1932, Lamson 1918). No breeds of cattle have been found to be immune to infestations of lice (Babcock & Cushing 1942, Matthysse 1946). This study was designed to gather information which might offer some explanation for variations in population density and host susceptibility. The cattle biting louse, *Bovicola bovis* (L.), on diary cattle was the principal species studied.

Materials and Methods.—Cattle used in this study were heifers at the University Farm. They were held in two large pens and were permitted to intermingle freely in the barn as well as in adjacent exercise lots. The animals were stanchioned only during feeding and it was at this time that they were examined for lice.

Twelve heifers—six Holsteins, two Gurenseys, two Jerseys and two Brown Swiss—were selected for a detailed study and were examined at weekly intervals during the winter. The examiner used a head lamp and a pair of stereoscopic magnifiers. The hair was parted with the fingers and an actual count made of lice in that area during a specific time interval. Five such counts were made in areas selected at random on the back, rump, shoulder, and belly of each animal. This procedure provided an estimation of the actual infestation of a given animal as compared with others in the same study. The total number of lice counted at any particular time would give an index of the population level at that time. The total number of lice counted on one animal during the season gave an indication of its susceptibility to infestation as compared with other animals in the same study. Specific methods used in this study will be described along with results.

Table 1.—Seasonal changes in louse population on 12 dairy heifers at Madison, Wis. 1957–58.

Date	Total Number of Lice Counted	Date	Total Number of Lice Counted
11/24	53	1/26	235
12/1	65	2/2	222
12/8	99	2/9	203
12/15	153	2/16	87
12/22	154	2/23	116
12/29	190	3/2	106
1/5	246	3/9	51
1/12	194	3/16	55
1/19	293		

Results.—The weekly counts, begun on November 24 and continued until March 16, are summarized in table 1.

The data indicated that the louse population began to increase following the November count and reached a peak in January. During the latter part of February and early March the population dropped quite rapidly.

Individual records were kept on each animal. Table 2, column 2, reveals the wide range of susceptibility to louse infestation. Holsteins ranged from 228 to 738, Brown Swiss from 12 to 45, Guernseys from 10 to 53, and Jerseys from 2 to 4. While numbers were very limited, these data support the claim that Holstein cattle are more susceptible to lice than the other dairy breeds.

As a means of determining causes for such differences in louse infestations, samples of hair were selected from comparable areas and the diameters were measured. Four hundred fifty hairs from each of the 12 animals were measured. The average diameters are summarized in table 2, column 3. Statistical analysis of these data revealed no significant difference in hair diameter between breeds of cattle.

The difference in diameter of hair within the Holstein breed, however, was highly significant. There was also a positive correlation between the louse population and hair diameter (significant at 5% level).

In a comparable host-parasite relationship (human head louse) the actual hair density was considered a limit-

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Table 2.—Louse population counts: average diameter and length of individual hair; density of hair per unit area of skin surface; and total residue, dry matter, and pH of skin secretions of cattle used in the study.

Ear Tag No.[a]	Louse Population	Diameter[b]	Density[c]	Length (mm.)	Residue[d]	Dry Matter[e]	pH
H489	434	4.116	26	37	441	326	5.50
H492	738	4.672	26	32	547	383	5.47
H494	228	3.902	23	31	463	284	5.54
H496	413	3.989	20	27	566	334	5.63
H497	358	4.153	28	30	556	375	5.60
H500	351	4.098	19	30	374	298	5.33
S51	12	3.580	14	30	471	299	5.53
S49	45	4.120	17	30	515	334	5.52
J104	4	3.200	21	26	323	295	5.51
J105	2	4.939	15	21	405	271	5.57
G134	10	3.720	24	27	462	285	5.48
G129	53	3.634	26	28	539	285	6.51

[a] H indicates Holstein; S, Brown Swiss; J, Jersey; and G, Guernsey.
[b] One unit equals 15.5 microns. Each value represents 450 measurements.
[c] The weight of the hair divided by the area in mm. from which it was obtained (×.001).
[d] Weight in .01 gram.
[e] Weight in .0001 gram.

ing factor in parasite distribution. When hair is clipped from cattle, it hangs together quite well and can be easily collected. The weight of the hair clipped from a small area divided by the area in millimeters from which it was clipped was used in this study as an index of its density. The average hair densities of the cattle studied is summarized in table 2, column 4. Though the differences in hair densities appeared to be rather great, these differences could not be correlated with louse populations.

Some of the clipped hair was also fastened to paper with transparent tape in order to facilitate measurement of its length. These data are summarized in table 2, column 5. Though the length of hair varied considerably, it was not correlated with louse population.

Skin secretions of the host may be a factor in population cycles of obligate parasites. Samples of skin secretions were collected at monthly intervals, employing a mixture (equal parts) of ethyl alcohol and diethyl ether. One hundred fifty ml. of solvent was used to wash a circular area of approximately 6 inches in diameter on the back of the animal. The solvent and dissolved skin secretions were recovered with 15 grams of absorbent cellulose and placed in wide-mouth jars. In the laboratory the absorvent cellulose was washed twice with 150 ml. of solvent, squeezed dry, and discarded. The solvent containing skin secretions was concentrated by evaporation, warmed to room temperature, and filtered. The filtrate was collected in amber-blake bottles and stored in the refrigerator for further analysis. Residues remaining on the filter paper were dried and weighed. The weights of the residues are summarized in table 2, column 6.

The amount of residue obtained from the samples could not be correlated with host's louse population. However, the amount of residue obtained from cattle during the summer months appeared to be greater than during the winter. These data are summarized in table 3, column 2.

The volumes of the filtrates containing secretions were then adjusted to 150 ml. Twenty-five ml. of this material was placed in a tared beaker and the solvent was evaporated off under an air stream. The beakers were then dried in an oven at 55° C. and weighed to determine the total dry matter. The total amounts of dry matter contained in 25 ml. of skin secretion aliquots are summarized in table 2, column 6. Analysis of the data indicated no significant difference in amount of secretions produced by individual cattle. The amounts of secretions produced on a monthly basis are summarized in table 3, column 3. These data indicated a greater amount of secretions produced during winter months than during the summer.

Table 3.—Residue, total dry matter, and pH of skin secretions of all cattle used in the study.

Month	Residue	Dry Matter	pH
January	206	300	5.94
February	198	226	5.42
March	202	262	5.95
April	299	272	5.64
May	415	296	5.24
June	655	240	5.47
July	714	222	5.35
August	670	214	5.22
September	682	225	5.45
October	797	623	4.97
November	570	468	5.42
December	254	419	6.12

Twenty-five ml. of distilled water was added to beakers containing dried skin secretions. After warming these mixtures and then cooling to room temperature, the pH of the mixture was determined. The pH readings are summarized in table 2, column 8. Analysis of the data indicated no significant difference in pH of the skin secretions between cattle. In table 3, column 4, the monthly pH readings are summarized. These data revealed that the pH of the skin secretions was lower during the summer months than in the winter.

When the skin secretions (extracts) were treated with sulfuric acid and acetic anhydride, a blue-green color was produced (Leiberman-Burchard reaction) indicating the presence of sterols. The optical densities for each breed of cattle were plotted on a graph as represented in figure 1. Differences in the curves were due to the differences in concentrations of skin secretion extracts rather than types of skin secretions.

The Schoenheimer-Sperry reaction was employed to determine the presence or absence of certain sterols in the skin secretions. The rate as well as the intensity of color changes is indicative of the type of compounds present. For this test the following technique was employed: Twenty-five ml. of the skin secretion extracts were placed in beakers and evaporated to dryness. The dried secretions were dissolved in 20 ml. of glacial acetic acid. A reagent consisting of 19 ml. of acetic anhydride and 1 ml. of sulfuric acid was prepared and allowed to adjust to room temperature. Four ml. of this reagent and 2 ml. of the acetic acid solutions containing skin secretions were placed in a beaker, thoroughly mixed, placed in a corex spectrophotometer cell, and readings were taken every minute for a period of 30 minutes. All readings were taken at a wave length of 660 mu.

When the skin secretions were dissolved in glacial

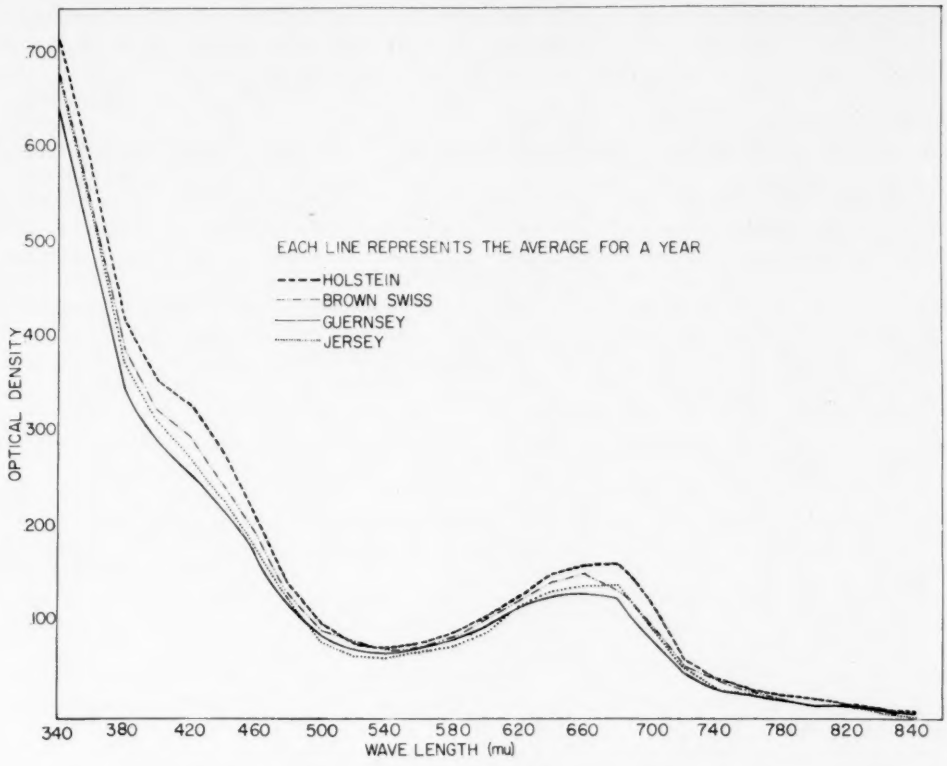

FIG. 1.—Absorption spectrum of skin secretion extracts.

acetic acid and treated with sulfuric acid and acetic anhydride, the color developed slowly. The optical densities for each breed were plotted on a graph (fig. 2). All the skin secretions responded in the same manner.

The response of the skin secretions to this test is in many ways similar to that of certain sterols. To further verify this observation, cholesterol was treated in the same manner as the skin secretions. A response was obtained which was somewhat similar to that of the skin secretions. Figure 2 illustrates the comparative responses of skin secretions and of cholesterol. Cholesterol reacted more slowly to the Schoenheimer-Sperry test than the skin secretions. Sterols such as 7-dehydrocholesterol and 7-OH cholesterol respond very rapidly to the same biochemical test (Moore & Bauman 1952).

DISCUSSION.—Evidence is presented that Holstein cattle were more heavily infested with lice than other breeds of cattle used in the same study. The skin of Jersey cattle is dark and, therefore, the lice are more difficult to see. However, this study also included Guernsey and Brown Swiss cattle. The skin of Guernsey cattle is light and they also possess white hair as do Holsteins. In no case, however, were there as many lice on Guernsey, Jersey, or Brown Swiss as there were on Holsteins. Referring to the original data of the work reported by Allen & Dicke (1952) it is found that similar breed differences were apparent. In a group of five Holsteins, two Jerseys, and two Guernseys, mingling freely, the Holsteins averaged 46.5 as compared with 10.2 for the other two breeds. In another group, with one side of the body clipped, the counts on the unclipped sides of five Holsteins averaged 65.8 as compared to 27.8 for two Jerseys and one Guernsey. In both groups, there was one Guernsey with a count above the Holstein average, and there were three Holsteins below the Jersey-Guernsey average. Individual differences within breed are apparent, and it is possible that under certain conditions Guernsey, Jersey, and Brown Swiss cattle may be very susceptible to louse infestation. Matthysse (1946) reported all breeds of cattle susceptible to louse infestation. His observations were made on a number of farms in New York. In this study all breeds of cattle were maintained under identical conditions, and mingling together freely eliminated the variations which normally occur between farms.

Weekly counts beginning in November indicated a gradual increase in louse population. This buildup continued to the latter part of January, after which the population declined. By May 1 all cattle appeared to be free of lice. Since the animals were kept in the feed lot on a uniform ration of mixed hay, silage, and grain until May 15, this eliminates the possibility that diet was associated with the louse population fluctuations as some workers have indicated.

Attempts were made to locate lice during the spring and summer months of 1954. Cattle were examined at monthly intervals and at no time were any lice found. It was noted that a great deal of skin scurf accumulates in the hair during the summer months. This is also reflected in table 2, column 6. It is very likely that some lice survive the summer heat in the skin scurf on shaded parts of the body. However, since the population is very low, it is difficult to locate them.

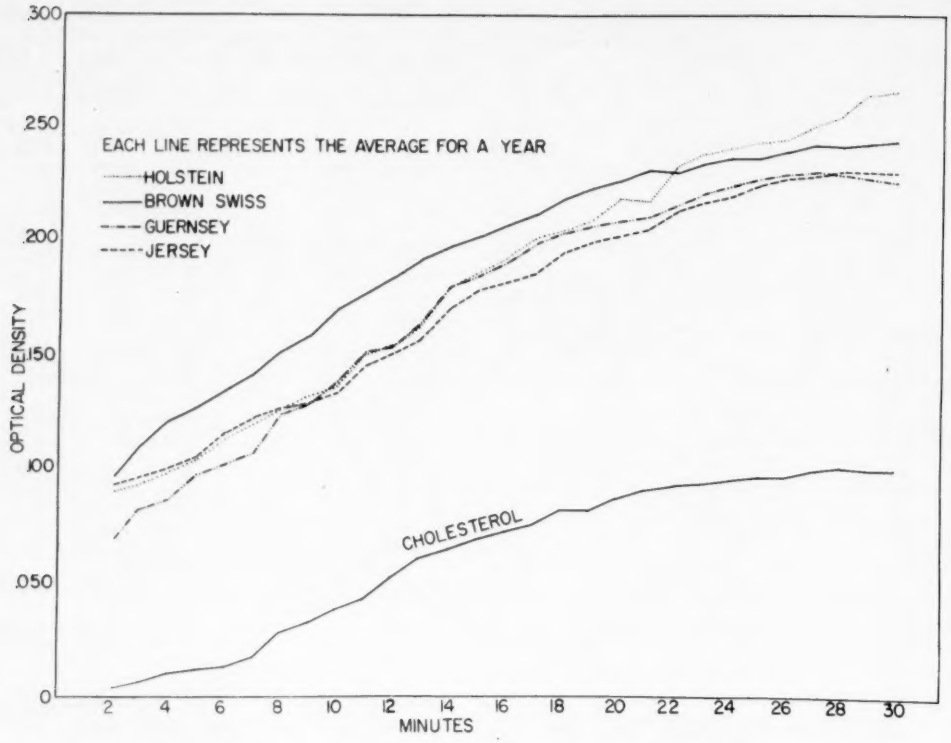

FIG. 2.—Response of skin secretion extract and cholesterol to the Schoenheimer-Sperry reagent.

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The Stability of Hydrogenated Gypsy Moth Sex Attractant[1]

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ABSTRACT

The gypsy moth sex attractant deteriorates rapidly, so that most of its original potency is lost when it is exposed during the next flight season, a year later. Eight lots of this attractant collected between 1947 and 1956 were stabilized by hydrogenation and tested periodically to determine their loss in attractancy when stored at 4° C. Results of these tests indicate that the hydrogenation procedure is effective in preventing deterioration of the lure. In the latest tests hydrogenated lures that were collected 9, 4, 3, 2, and 1 years previously were equally effective. Hydrogenation should be carried out shortly after collection of the lure. Details of this procedure are given.

A means of stabilizing the activity of the female gypsy moth (*Porthetria dispar* (L.)) sex attractant by hydrogenation was reported by Haller *et al.* (1944). The purpose of the present communication is to report on the details of the latest hydrogenation procedure, on the effectiveness of this stabilization process, and on related aspects of this problem. Tests conducted periodically over the last 10 years indicate that the hydrogenation process is effective.

The adult female moth, which does not fly, emits a material[3] that lures the flying male to it for mating. Mating takes place only once a year, usually during a 10-day period in July and August when the male moths are in flight. Traps are baited with the attractant to scout vast

[1] Accepted for publication July 30, 1958.

[2] Retired.

[3] Studies on the isolation and chemistry of this attractant, named gyptol, have been reported by Acree (1953a, 1953b, 1954).

areas for the presence of the moth, and control measures are applied where the insect is found. This procedure has been especially useful in pushing back the fringes of an infestation, and thereby limiting further spread of the insect.

At first traps were baited with live females (Collins & Potts 1932), but the possibility of their escaping in uninfested areas forced the discontinuance of this procedure. Traps baited with the last two abdominal segments of the female, hereafter called tips, are effective for an exposure period of 6 weeks when the tips (or their extract) are fresh; but the attractant deteriorates rapidly, even under refrigeration, so that it is far less effective when exposed during the next flight season, a year later. In order for control workers to conduct an effective program, it was necessary to stabilize the attractant so that it could be collected and stored in advance, particularly during those years when the supply was plentiful. Such a stabilization procedure is given below.

PROCESSING OF GYPSY MOTH TIPS FOR STABILIZATION OF LURE.—*Extraction.*—Bulk collections of gypsy moth tips are made in thiophene-free benzene, because thiophene will poison the catalyst used in the hydrogenation process. The tips are usually processed within a month or two after collection. Two clean cloth bags ($11\frac{1}{2}\times19$ inches when laid flat), one inside the other, are placed in an 8-quart fruit press and loaded with the material. The tops of the bags are folded over and the tips compressed. The filtrate runs into a 10-gallon carboy. Toward the end of the pressing an aqueous emulsion is expelled and forms a layer under the benzene. The bag of tips is removed from the press and placed in a bucket, which is then half-filled with benzene. After having been kneaded, the tips are returned to the press along with the benzene wash and pressed again. After two more washings in a similar manner to extract practically all the attractant, the tips are discarded.

The benzene layer is transferred to a still for the removal of the solvent. The aqueous layer that remains is diluted with 4 volumes of water, gently mixed with 1 volume of benzene, and allowed to stand overnight. The nonemulsified aqueous layer is discarded. The mixture is gently shaken with more water and generally clears after standing for a few hours; otherwise the procedure is repeated. The benzene layer is separated and, if curds remain, it is filtered through two dry cloth bags in the fruit press and then added to the main batch.

This procedure is repeated until 100,000 tips are extracted, a total of 18 to 25 gallons of the combined benzene filtrate and wash being obtained. *Caution!* Benzene vapors are toxic. These operations should be carried out under adequate ventilation, and solvent-resistant gloves should be worn.

Concentration of Extract.—The benzene layer is rapidly evaporated below 50° C. at reduced pressure in a large laboratory evaporator.[4] Batches of 5 gallons are run through in 40 to 45 minutes. The volume obtained from 100,000 tips is reduced to 2 to 3 liters, and this product is filtered.

Final Evaporation.—Evaporation of the concentrate by conventional means is slow and therefore subjects the attractant to prolonged heating. In a large rotating evaporator[5] fitted with a 2-liter flask, the extract from 100,000 tips is concentrated quickly under reduced pressure to about 200 to 350 ml. on a 45° C. water bath. When the manometer shows 50 to 70 mm. pressure, the evaporation is discontinued. The purpose of this evaporation is to remove almost all the benzene, so that the hydrogenation of the extract may be carried out in ethyl acetate.

Hydrogenation.—The extract is hydrogenated in a 2-liter long-neck, round-bottom flask with an apparatus similar to that used by Adams & Voorhees (1941). The hydrogen is bled into the flask from a 4-liter tank, and the pressure of the gas in the flask is maintained between atmospheric and 5 cm. Hg. above (manometer in the system); the hydrogen uptake is measured by the pressure drop of the gauge on the tank.

By means of a long-stemmed powder funnel a slurry of 2 grams of platinum oxide (Adams *et al.* 1941) in ethyl acetate is introduced into the flask and washed in with about 25 ml. of the solvent. The flask is connected to the hydrogen source and is alternately evacuated and filled with hydrogen three times. (Air must be excluded or an explosion may result.) The flask is shaken until the catalyst is reduced, and the extract of 100,000 tips is then washed in with 200 to 250 ml. of ethyl acetate. Again hydrogen is introduced after the flask has been flushed three times, and the hydrogenation (exothermic) is continued until there is no further uptake (about 3 hours). The uptake of hydrogen is usually 7 to 12 liters. The golden-brown attractant material turns dark green or almost black on hydrogenation. If the catalyst becomes deactivated (little or no uptake of hydrogen), more catalyst, slurried in ethyl acetate, may be added without filtering off the old catalyst. To be certain the material is sufficiently hydrogenated, another gram of catalyst may be added. If little further uptake of hydrogen occurs on shaking, the hydrogenation step is complete.

Filtration.—Since the hydrogenated extract tends to solidify, the reaction mixture must be warmed and diluted with benzene to permit filtration. The catalyst is collected on a 9-cm. Büchner funnel and washed with benzene. The filtrate is stored at 4° C. until needed. *Caution!* Keep catalyst on the filter covered with solvent; if allowed to dry, it may ignite.

STABILITY TESTS.[6]—These tests were conducted between 1949 and 1957. The attractant material was the hydrogenated extract obtained from pupae collected at different locations in New England except for some material collected in Spain and Yugoslavia in 1956. Traps baited with the lure (usually the extractive equivalent to approximately 15 tips per trap) were set out in the New England area just prior to the flight of the male moths. They were arranged in four replications of a randomized-block design. They were placed 4 feet high on trees about 10 to 15 feet apart, and blocks were separated from each other by at least 400 feet. Catches were usually checked every other day during the flight season.

The catches obtained for each material are given in table 1. Most of the tests were continued until the catch of moths was very low, but in 1956 the catches were so

[4] No. 5486, Precision Scientific Co., Chicago, Ill., connected in series with a No. 6341 heat exchanger, Graham Mfg. Co., New York, N. Y.

[5] Rinco Instrument Co., Greenville, Ill.

[6] M. George and E. D. Eckess, of the Plant Pest Control Division, assisted in conducting the field tests.

Table 1.—Effect of storage at 4° C. on the activity of hydrogenated gypsy moth sex lure (15 tips per trap).

Year Tested	Year of Collection	Years Stored	Number of Males Captured					
			Block				Total	Mean
			A	B	C	D		
1949	1947	2	3	6	8	25	42	10.5
	1948	1	10	8	6	6	30	7.5
1950	1947	3	68	101	87	90	346	86.5
	1948	2	36	22	43	52	153	38.25
1951	1947	4	19	17	21	35	92	23
	1948	3	45	46	58	58	207	51.75
	1950	1	5	12	6	5	28	7
1952	1947	5	80	42	71	50	243	60.75
	1948	4	52	83	75	75	285	71.25
1953	1947	6	95	81	72	97	345	86.25
	1948	5	104	80	90	81	355	88.75
1955	1947	8	13	17	17	29	76	19
	1948	7	33	19	25	33	110	27.5
	1953	2	39	29	32	44	144	36
	1954	1	47	30	39	47	163	40.75
1956	1947	9	199	137	94	88	518	129.5
	1948	8	259	171	103	111	644	161
	1953	3	255	160	112	130	657	164.25
	1954	2	177	174	140	130	621	155.25
	1955	1	383	183	142	153	861	215.25
1957	1947	10	7	13	21	13	54	13.5
	1948	9	31	93	57	39	220	55
	1953	4	44	53	58	50	205	51.25
	1954	3	17	64	73	48	202	50.5
	1955	2	24	51	71	55	201	50.25
	1956	1	3	58	49	94	204	51
	1956[a]	1	24	17	29	29	99	24.75
	1956[b]	1	9	44	37	55	145	36.25

[a] Collected in Spain.
[b] Collected in Yugoslavia.

heavy that tests were discontinued when catches were at their peak.

ANALYSIS OF RESULTS.—The variation in potency of the lures is due to the degree of infestation, the health of the insect, processing procedures, and other factors, some beyond experimental control. Since no standard was available for comparison of the lures collected in different years, it was difficult to make exact determinations of deterioration with age of storage. However, since the 9- and 10-year-old materials were still active in the latest tests, it may be concluded that deterioration of the hydrogenated lure in storage takes place very slowly.

The 1947 lure may have deteriorated more than the 1948 lure, since in the 1949 and 1950 tests its captures exceeded those of the 1948 material whereas in all subsequent tests its captures were lower. A statistical analysis was therefore made to determine whether the differences obtained each year between the 1947 and 1948 materials were significant. The F values calculated for each test year follow:

Year of Test	*F*
1949	0.28 n.s.
1950	20.97*
1951	91.83**
1952	0.50 n.s.
1953	0.12 n.s.
1955	4.36 n.s.
1956	8.52 n.s.
1957	10.04 n.s.

* $F_{0.05} = 10.129$.
** $F_{0.01} = 34.117$.

The data show much variation, but from the overall picture the 1947 lure appears to be deteriorating as compared with the 1948 material; in 1950 the 1947 material was significantly better, but in 1951 the 1948 material was significantly better. Furthermore, the trend of the F values in the 1953 to 1957 tests seems to show a steady decline in activity for the 1947 lure. In contrast to this behavior, the 1948 material does not appear to be losing activity, since in the 1956 and 1957 tests its captures are in the same range as those of materials collected 5 to 8 years later. The possibility that the 1948 material gained potency in storage is not considered likely.

Delay in hydrogenation after collection is detrimental to the stabilization of the lure. Present indications are that the greater the delay, the less stable the lure will be. The lures collected during the summers of 1951 and 1952 were processed in the winter of 1952–53—*i.e.*, $1\frac{1}{2}$ and $\frac{1}{2}$ year after collection. In the tests that followed in the summer of 1953, the 1951 material was practically inactive but the 1952 lure was active. However, in tests during subsequent years the 1952 material exhibited little activity. It must be concluded, therefore, that even a delay of a half-year may seriously affect the stability of the product. The hydrogenation of the 1947 lure was delayed 4 months longer than that of the 1948 material, and this delay may account for its lower stability. The 1953, 1954, 1955, and 1956 lures were hydrogenated as soon as practicable after collection, and these materials have shown no signs of deterioration.

Attractants whose hydrogenation was delayed absorbed less hydrogen than those processed without delay. It has been further speculated that the amount of hydrogen absorbed by a lure might roughly parallel its activity. In 1956 domestic, Spanish, and Yugoslavian materials were hydrogenated, and they absorbed about 0.11, 0.075, and 0.09 ml. of hydrogen per tip, respectively. The activity of these lures, when tested in 1957) (table 1), paralleled their hydrogen uptake, a result that lends credence to the hypothesis.

The effect of the health of the insect on the potency of its lure was indicated with the lure collected in 1950, when the gypsy moth encountered widespread disease and parasitism. That year trained personnel were able to collect only 91,000 pupae from which only 13,100 tips (14.5% emergence) were obtained. Although this small amount of lure was processed without delay, it proved to be very weak when exposed in the 1951 test lines (table 1). The number of pupae collected and the percentage of tips obtained between 1947 and 1955 are shown below:

Year	*Pupae Collected*	*Percentage of Tips Obtained*
1947	1,336,680	27.4
1948	1,815,200	56.5
1950	91,000	14.5
1951	479,350	38.2
1952	1,275,000	37.9
1953	1,661,000	53.7
1954	556,700	43.6
1955	105,000	43.8

When an analysis of variance was made of the tests run during 1956 and 1957 (table 1), the mean captures proved by the F test to be significantly different for the 1957 data and almost significantly different for the 1956 data. In the 1957 tests the least significant difference between any two means was 24.6 at the 5% and 33.55 at the 1% level. The test results between the 1948, 1953, 1954, 1955,

Table 2.—Effect of lure concentration on male moth captures during 1957.

Year of Collection	Number of Tips per Trap	Number of Males Captured					
		Block					
		A	B	C	D	Total	Mean
1947	1	0	1	3	2	6	1.5
	2	6	5	15	2	28	7
	4	18	5	9	3	35	8.75
	15	7	13	21	13	54	13.5
1956	1	9	3	5	20	37	9.25
	2	28	15	15	15	73	18.25
	4	9	18	29	24	80	20
	15	3	58	49	94	204	51

and 1956 domestic materials were therefore not significantly different from each other, but the difference between the 1947 mean and that of any other domestic material was highly significant. Of the two foreign lures only the Spanish material was significantly weaker than the domestic materials.

In the 1956 tests the 1947 attractant was again the weakest, but the difference in activity between it and the 1948, 1953, and 1954 materials was not significant, as the least significant difference between means was 55.6; the least significant difference at the 1% level was 78. The difference between the 1947 and 1955 lures was highly significant.

The foregoing analysis of data is based on the method of assay being valid. A practical verification of this point was made by exposing in 1957 four different tip streng ths of the weakest (1947) and four of the most active (1956) materials. The results, recorded in table 2, show that the concentration of lure (tips per trap) does parallel captures. Substantiation of the assay does not fall within the purview of this paper, but many experiments were run in which tip strength was varied, and results indicated that tip strength does parallel captures but increasing the tip strength above 16 does not result in significantly greater captures. Because of this finding traps used in the field for survey work have been baited with 12 to 16 tips each.

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Toxicity of the Piperonyl Ester of Chrysanthemumic Acid and Some of Its Derivatives in Comparison with Allethrin and Pyrethrins as House Fly Sprays[1]

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Abstract

Eighteen related synthetic compounds, most of them substituted piperonyl esters of *dl-cis-trans*-chrysanthemumic acid, were tested as space sprays on the Campbell turntable against house flies, *Musca domestica* L. The most toxic were the 6-bromo- and 6-chloropiperonyl esters, the latter now known as barthrin. They were, respectively, about 21 and 31% as toxic as allethrin, and 51 and 83% as toxic as pyrethrins at the 50% mortality level. Above this level the ratios were higher, reaching at the 95% mortality level to about 25 and 50%, respectively, as toxic as allethrin, and 90 and 200% as toxic as pyrethrins. The knockdown value of the esters, although excellent at highly toxic concentrations, was much inferior to that of allethrin or pyrethrins at comparable concentrations.

In screening tests on a number of compounds synthesized by Barthel and coworkers (Chen & Barthel 1956, Barthel & Alexander 1958), Piquett & Gersdorff (1958) found the piperonyl ester of *dl-cis-trans*-chrysanthemumic acid and several of its derivatives to be toxic to house flies (*Musca domestica* L.). Earlier tests with some of the compounds against several species of insects were reported by Chen & Barthel (1956), and tests showing the piperonyl ester itself to be toxic were reported by Synerholm (1949), and Inoue *et al.* (1951). Tests with related compounds were reported by Barthel & Alexander (1957). This paper reports further tests with some of the esters screened against house flies.

MATERIALS.—Pyrethrins and allethrin were used as standards of comparison. The sample of pyrethrins was the unrefined extractive in which 54.2% of the total pyrethrins consisted of pyrethrin I and cinerin I as determined by the mercury-reduction method. The sample of allethrin was of 93.4% purity as determined by the hydrogenolysis method.

After preliminary tests, sprays of the more toxic esters, including the standards, were prepared in refined kerosene

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(Deobase) at concentrations selected to cause a range of mortalities from low to high. Sprays of the esters of low toxicity were prepared in the same solvent at a single, relatively high concentration.

KNOCKDOWN AND MORTALITY DATA.—Knockdown and mortality of house flies of the National Association of Insecticide and Disinfectant Manufacturers' 1948 strain were determined in replicated tests by the Campbell turntable method. The tests were made in four series, a series comprising the data pertaining to the same group of populations of flies. All sprays in a series were tested simultaneously and but once on each population in the group. The data are summarized in table 1.

Table 1.—Knockdown and mortality of house flies caused by kerosene sprays containing certain new synthetic esters, allethrin, and pyrethrins.

MATERIAL	CONCENTRATION (MG. PER DL.)	PER CENT	
		Knockdown in 25 Minutes	Mortality in 1 Day
Series 1. Eight replicates, 106 flies each			
Chrysanthemumic acid, 6-bromopiperonyl ester	1012.5	98.3	97.9
	675.0	95.3	89.5
	450.0	64.6	67.1
	300.0	19.1	31.6
	200.0	11.4	10.7
	133.3	6.4	3.4
Allethrin	214.1	100	93.4
	142.8	100	84.0
	95.17	100	71.2
	63.45	99.8	39.5
	42.30	99.1	14.9
	28.20	95.8	4.0
Pyrethrins	759.4	100	94.0
	506.3	100	89.7
	337.5	100	72.7
	225.0	100	60.3
	150.0	100	40.6
	100.0	100	21.6
Series 2. Six replicates, 107 flies each			
Chrysanthemumic acid, 6-chloropiperonyl ester	666.7	100	99.8
	444.4	99.8	97.4
	296.3	98.3	78.5
	197.5	92.0	52.4
	131.7	80.6	16.5
	87.79	63.3	3.8
Cyclopropanecarboxylic acid, 2-isobutyl-3,3-dimethyl-, piperonyl ester	2000	100	100
	1333	99.8	96.4
	888.9	97.4	73.1
	592.6	79.1	35.5
	395.1	71.9	5.1
Allethrin	214.1	100	93.2
	142.8	100	85.4
	95.17	100	71.3
	63.45	100	53.3
	42.30	99.7	32.6
	28.20	99.8	12.1
Pyrethrins	759.4	100	91.9
	506.3	100	88.0
	337.5	100	77.6
	225.0	100	59.1
	150.0	100	49.9
	100.0	100	28.7
	66.67	100	19.0
Series 3. Six replicates, 102 flies each			
Chrysanthemumic acid, α-allylpiperonyl ester	3000	1.0	0.5
α-(1,1-dimethylpropyl)piperonyl ester	2000	97.3	67.1
	1333	72.9	32.0
	888.9	41.0	11.1
	592.6	27.9	2.3
α-isopropylpiperonyl ester	3000	5.6	1.1
α-methylpiperonyl ester	3000	30.0	36.2
	2000	7.6	8.1
	1333	5.2	1.0
α-pentylpiperonyl ester	3000	17.4	0.6
piperonyl ester	1333	100	92.7
	888.9	98.9	69.9
	592.6	95.0	31.2
	395.1	48.4	8.7
	263.4	28.8	1.0
α-propylpiperonyl ester	3000	8.8	1.7
Allethrin	214.1	100	81.3
	142.8	100	69.9
	95.17	100	49.4
	63.45	100	22.6
	42.30	99.1	7.4
	28.20	97.4	1.4
Pyrethrins	759.4	100	86.5
	506.3	100	77.0
	337.5	100	56.8
	225.0	100	34.6
	150.0	100	20.3
	100.0	100	5.9
	66.67	99.6	2.7
Series 4. Three replicates, 102 flies each			
Chrysanthemumic acid, α-butylpiperonyl ester	3000	0.4	1.0
α-*tert*-butylpiperonyl ester	3000	0.3	1.0
α-cyclohexylpiperonyl ester	3000	0	0
α-methallylpiperonyl ester	3000	—[a]	1.9
3,4-methylenedioxybenzhydryl ester	3000	0	1.8
4-(3,4-methylenedioxyphenyl)-*sec*-butyl ester	3000	0	0.7
α-phenethylpiperonyl ester	3000	0	0.7
6-propylpiperonyl ester	3000	0	1.0
Allethrin	214.1	100	77.0
	142.8	100	60.7
	95.17	100	35.7
	63.45	100	19.1
	42.30	99.6	4.5
	28.20	98.5	1.6
Pyrethrins	759.4	100	88.7
	506.3	100	74.9
	337.5	100	58.8
	225.0	100	47.6
	150.0	100	26.1
	100.0	100	16.8
	66.67	100	7.2

[a] Knockdown is not given because solvent is 1:1 acetone-Deobase.

EVALUATION OF RELATIVE TOXICITY.—To evaluate relative toxicity and to determine the precision of the estimates, the mortality data for those materials tested at more than one concentration in series 1 to 3 were subjected to probit analysis as described by Finney (1952). Provisional individual regression lines were fitted. It appeared that the data for the piperonyl esters were represented by lines of steeper slope than those for allethrin and that those for the new esters in series 3 could be adequately represented by parallel lines. The statistical procedures based on these hypotheses were followed to obtain the weighted regression equations, and subsequent analysis of chi-squares gave no evidence that the data could not be so represented. However, heterogeneity factors were required in the calculation of variances in some of the analyses.

The final equations showing the regression of mortality, expressed in probits, on concentration in milligrams per deciliter, expressed as logarithms, are given below. Heterogeneity factors (H.F.) and the standard errors (s_b) of the regression coefficients are also given.

From these equations LC-50's were determined and evaluations of relative toxicity were then obtained as the

		s_b	H.F.
Series 1:			
Chrysanthemumic acid, 6-bromopiperonyl ester	Y=4.5441X− 6.6479	0.147	2.454
Allethrin	Y=3.7451X− 2.0390	.289	9.080
Pyrethrins	Y=2.6920X− 1.1230	.122	2.668
Series 2:			
Chrysanthemumic acid, 6-chloropiperonyl ester (barthrin)	Y=5.1547X− 6.8486	0.242	2.671
Cyclopropanecarboxylic acid, 2-isobutyl-3,3-dimethyl-, piperonyl ester	Y=6.2967X−12.9019	.199	1
Allethrin	Y=2.9797X− 0.3534	.087	1
Pyrethrins	Y=2.2598X− 0.0113	.102	2.345
Series 3:			
Chrysanthemumic acid, α-(1,1-dimethylpropyl) piperonyl ester	Y=5.2715X−11.8897	0.206	2.875
α-methylpiperonyl ester	Y=5.2715X−13.7284	.206	2.875
piperonyl ester	Y=5.2715X−10.0299	.206	2.875
Allethrin	Y=3.4303X− 1.9399	.250	5.851
Pyrethrins	Y=2.9776X− 2.3796	.079	1

inverse ratio of the pertinent pair of LC-50's. The estimations together with their standard errors are given in table 2.

Because the regression lines for the piperonyl esters are of greater slope than those for allethrin, and therefore those for pyrethrins, ratios of comparison with these standards will vary between low and high mortality levels. Such ratios were estimated from LC-5's and LC-95's determined directly from the equations and are also given in table 2 to indicate the approximate range possible.

DISCUSSION.—The five most toxic of the new chrysanthemumic acid esters were the 6-chloropiperonyl, 6-bromopiperonyl, piperonyl, the α-(1,1-dimethylpropyl) piperonyl, and α-methylpiperonyl esters; they were, respectively, about 31, 21, 15, 7, and 3% as toxic as allethrin and about 83, 51, 42, 19, and 8% as toxic as pyrethrins at the 50% mortality level. Above this level the ratios were higher, reaching with the most effective compounds, the bromo and chloro derivatives, at the 95% mortality level to about 25 and 50%, respectively, as toxic as allethrin, and to 90 and 200% as toxic as pyrethrins. Thus, the two halogenated esters, especially the chloro, are worthy of further investigation as insecticides, not only because of their relatively simple structure but also because of their high toxicity.

The piperonyl ester of 2-isobutyl-3,3-dimethylcyclopropanecarboxylic acid possessed appreciable toxicity, being about 5, 9, and 18% as toxic as allethrin at the 5, 50, and 95% mortality levels, respectively, and 8, 24, and 69% as toxic as pyrethrins. This compound differs structurally from the piperonyl ester of chrysanthemumic acid in that the double bond in the acid component has been saturated. When such a change was made in the allethrin molecule, the toxicity of the ester was decreased to about one-third (LaForge *et al.* 1952). In the present comparison the toxicity of the ester is decreased to about one-half. Again it is shown that the presence of this double bond, though of marked importance in the toxic action of such compounds, is not a prerequisite to toxicity.

All the toxic esters possessed knockdown value, those concentrations which caused very high mortality causing practically complete knockdown. However, at comparable

Table 2.—Toxicity relative to allethrin and pyrethrins of some new synthetic esters.

MATERIAL	SERIES	LC-50 (MG. PER DL.)	PER CENT TOXICITY RELATIVE TO—[a]		PER CENT RANGE IN RATIO TO—[b]	
			Allethrin	Pyrethrins	Allethrin	Pyrethrins
Chrysanthemumic acid, 6-bromopiperonyl ester	1	365.9 ± 6.5	20.71±0.94	51.4 ±1.8	17.3−24.7	29.0− 91.2
6-chloropiperonyl ester	2	198.9 ± 4.1	31.48±0.87	83.0 ±3.3	18.4−53.8	32.4−213
α-(1,1-dimethylpropyl) piperonyl ester	3	1599 ± 40	6.60±0.32	18.82±0.58	4.5− 9.7	10.8− 32.7
α-methylpiperonyl ester	3	3571 ±120	2.95±0.16	8.43±0.32	2.0− 4.3	4.8− 14.7
piperonyl ester	3	709.8 ± 15.9	14.86±0.71	42.4 ±1.2	10.1−21.9	24.4− 73.8
Cyclopropanecarboxylic acid, 2-isobutyl-3,3-dimethyl-, piperonyl ester	2	696.7 ± 8.1	8.99±0.20	23.67±0.86	4.6−17.6	8.1− 69.4
Allethrin	1	75.77± 3.17	100	248 ±13	100	167 −369
	2	62.61± 1.14	100	264 ±10	100	176 −395
	3	105.5 ± 4.44	100	285 ±13	100	241 −338
Pyrethrins	1	188.2 ± 5.6	40.3 ±2.1	100	59.8−27.1	100
	2	165.0 ± 5.7	37.9 ±1.5	100	56.9−25.3	100
	3	300.9 ± 5.4	35.1 ±1.6	100	41.5−29.6	100

[a] At the 50% mortality level.
[b] Between the 5 and 95% mortality levels.

concentrations the knockdown value of the esters was much inferior to that of allethrin or pyrethrins.

The remaining 12 esters, showing practically no toxicity at the single, relatively high concentration at which tested, were, if toxic at all, less than one-eightieth as toxic as allethrin and less than one-thirtieth as toxic as pyrethrins.

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Relation of Oil Type, Deposit, and Soaking to Effects of Spray Oils on Photosynthesis in Citrus Leaves[1]

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ABSTRACT

This investigation concerns the effects of varying hydrocarbon composition and molecular size of spray oils on inhibition of photosynthesis in citrus leaves and their recovery with time after application. Effects were measured by determining regressions of inhibition of photosynthesis on amount of oil applied. Preselected known deposits of oil were applied to potted, single-leaf, rooted stem cuttings by means of a precision vertical spraying tower. Photosynthesis was measured by the Warburg procedure using a modified apparatus equipped with lights and temperature control bycmeans of either refrigeration or heat.

No sonsistent inhibition of photosynthesis of Eureka lemon or Bearss lime leaves occurred with California light-medium or medium-grade spray oils at the deposit level of 150 μg. oil/sq. cm. of leaf surface, an amount sufficient for the control of California red scale and citrus red mite.

Since the plants in the laboratory experiments were kept in the glasshouse they were not exposed to the ultra-violet components of sunlight. Data taken with leaves from trees sprayed in the field support the conclusion that ultra-violet light is not an important factor in the effect of spray oil on the photosynthesis of citrus leaves following application of amounts of oil necessary to control citrus pests.

A definite relation between inhibition of photosynthesis and increasing oil deposit was found in the deposit range of 300 to 600 μg. oil/sq. cm. of leaf surface; however, the data showed that the response to small increments of oil would be slight.

Recovery of photosynthesis occurred in less time in plants treated with napthenic oils than in those treated with paraffinic oils.

The principal effect on photosynthesis occurs in the tissue of the leaf marked by the dark discoloration known as oil soaking. During the first week after application, photosynthesis was inhibited 50 to 60% in the oil-soaked tissue. Tests with the tetrazolium reaction showed that the cells of the discolored tissue are not killed. Inhibition of photosynthesis seems to be the result of interference with gaseous exchange caused by the presence of the spray oil. Dissipation of the oil is accompanied by recovery of photosynthesis with a $\log_{10}$ relation to time.

The general effects of petroleum oil spray on photosynthesis in citrus leaves have been reported in a paper by Wedding *et al.* (1952). Relation of the investigation to its importance to citriculture in southern California was documented. Potted plants were sprayed with an aqueous mixture containing 2% of a representative California medium-grade proprietary emulsive spray oil. Determinations of photosynthesis were made by the Warburg method and were taken at a temperature of 29° C. Discs of leaf tissue put in the flasks were moistened with a slight film of water to hold them in place. Photosynthesis and respiration were depressed significantly in Washington Navel orange leaves the day following application of the oil, but recovery occurred with time. In Eureka lemon leaves a similar effect was observed for photosynthesis, but respiration was affected only slightly, and the depression of rate was not significant. Recovery of photosynthesis occurred sooner in lemon plants than in orange plants.

During studies of herbicidal action, Minshall & Helson (1949) observed that the effect of a light petroleum fraction on photosynthesis and respiration varied with the species of plant; in some plants disruption of function con-

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tinued until the plant was dead, while in others it was temporary and subsequent recovery was complete.

A fundamental concept of the phytotoxicity of hydrocarbon oils was developed by van Overbeek & Blondeau (1954), namely, that the semipermeability of the plasma membrane is influenced by the hydrocarbon molecule in accord with the phenomenon of solubilization. Within any configuration series (aromatic, paraffin, etc.) of increasing size, the smaller molecules are more toxic than the larger because the smaller molecules disrupt the plasma membrane more than the larger ones do. The evidence presented indicates that no solubilization or only a very low order of solubilization would occur with molecules as large as those of foliage spray oils. However, since solubilization is temperature-dependent, the action could be intensified as temperature increased and the curve of response shifted toward larger size. Van Overbeek & Blondeau (1954) observed that the range was very narrow in the relation of molecular size to effect on the semipermeability of the plasma membrane, being very close to an all-or-none response. Solubilization as the mode of phytotoxic action of hydrocarbon oils seems to agree well with experience in the use of foliage spray oils on citrus in southern California.

Van Overbeek & Blondeau (1954) applied the theory of solubilization to the explanation of the effects of oil on photosynthesis and respiration. They developed the idea that the presence of a hydrocarbon oil in plant tissue may have independent effects on the functions of photosynthesis and respiration, and that interference with photosynthesis may occur without accompanying disruption of respiration.

Penetration of petroleum oil from the surface into the tissue of citrus leaves was noted and studied as early as 1903 by Volck (1903). The oils were relatively wide fractions known as distillates and were not refined by the sulfonation treatment which was discovered later (Gray & deOng 1926). Retardation of the penetration of spray oils with a minimum unsulfonated residue of 92% into citrus leaves was observed by Knight & Cleveland (1934) when glycerol mono-oleate was dissolved in the oil to serve as an emulsifying agent for the spray mixture; they discuss the roles of viscosity and surface tension in the penetration of oil into leaf tissue. Ebeling (1939) reported the results of extensive studies on the penetration of spray oil into citrus leaf tissue. He found a range in the amount of retardation obtained with each of several solutes in the oil. Rohrbaugh (1941) concluded that penetration into a citrus leaf would continue as long as there was free oil on the surface of the leaf. If enough oil penetrates into a citrus leaf, a distinct dark discoloration will appear along the margin and midrib. Growers and spray operators call this condition "oil soaking." Ebeling (1950, p. 193) shows differences in amounts of discolored leaf area caused by application of excessive amounts of oil and of conventional spray mixtures (1.75% oil).

Evidence that spray oil dissipates from citrus foliage was reported by Cressman (1941) and by Ebeling (1950, p. 180). Cressman observed the relatively rapid loss of 29% of the deposit from lemon foliage during the first afternoon after spray treatment with light-medium oil in October.

In southern California a major consideration in the use of oil spray for citrus pest control is the potential effect on yield or juice quality of the fruit as a result of interference with the physiological function of the foliage by the oil applied. The intensive investigations of Chapman *et al.* (1943) and Pearce *et al.* (1952) demonstrated the relation of physical and chemical properties and of structural composition of petroleum oils to pest control efficiency. It appeared that the concepts they developed might have an application to the inhibition of photosynthesis of citrus leaves (Wedding *et al.* 1952) by spray oil.

The experiments reported here were planned to investigate the effects of varying hydrocarbon composition and molecular size on the inhibition of photosynthesis in citrus leaves, and recovery with time after application. Correlation of response with amount of oil applied seemed to be a desirable approach to the measurement of the effects of various oils. In addition, the correlations provided a calculated mean estimate of the threshold of inhibition of photosynthesis vs. amount of oil deposit.

MATERIALS AND METHODS.—The time intervals of the observations planned, and the function of the plant in the dissipation of spray oil, clearly indicated application of the treatments to living plants. Selected known deposits of oil could be applied to single leaves by means of a spray tower. To meet these requirements a technique for propagating single-leaf, rooted stem cuttings was developed.

Foliage for the stem cuttings was taken from Eureka lemon and Bearss lime trees which had never been treated with oil spray or other chemical pesticides. Stem material of the second growth cycle, *i.e.*, of the cycle preceding the terminal leaves at the time of cutting, was used. The second growth-cycle section of the stem can be distinguished readily by the characteristic appearance of the bark. It bears the young, vigorous mature leaves. This type of foliage seemed to be the best source of comparable samples of fully expanded leaves which had matured completely on trees under orchard conditions as successive sets of plants were needed during the course of the experiments.

Stem cuttings approximately 2 inches long, with a single leaf at the upper end of each, were taken. Only well-formed representative leaves without defects of any kind were kept. Procedures for rooting, potting, and care in the glass house were similar to those described by Riehl *et al.* (1958). Four-inch pots were used.

The oils used are described by the selected properties listed in table 1. Oils I and J were obtained from stocks of highly paraffinic composition. Although determinations were not made for percentages of paraffins of these oils, the values for gravity and viscosity index show the high paraffinicity. The isoparaffinic hydrocarbon oils K and L are fractions of an oil obtained from the process of catalytic alkylation of low molecular weight olefins. Isoparaffinic composition is evident in the relatively high viscosities for the fractional distillation ranges.

A modified (Wedding *et al.* 1952) 14-unit Warburg bath was used in these studies. Two manometers were used to measure thermo-barometric changes, and two were assigned to untreated check, leaving 10 manometers available for oil-treated tissue. Therefore, we decided to use 10 single levels of an increasing series of oil deposits for the regression of inhibition of photosynthesis. Determinations

Table 1.—Selected properties of experimental oils.

Type of Oil	Temperature, °F., for Distillation, Per Cent 10	50	90	Viscosity Saybolt 100° F.	210° F.	A.P.I. Gravity, 60° F.	Per Cent U. R.	Per Cent Paraffins by Waxman (aniline point)
California Spray Oil								
Light-medium grade								
A	572	624	684	67.5	35.3	29.3	94.8	51
B	583	626	693	72.5	35.8	27.7	93.0	50
C	581	626	707	67.4	35.5	30.8	94.8	59
D	572	623	714	72.0	35.7	28.6	92.2	51
Medium grade								
E	590	637	690	75.7	36.2	28.9	95.2	53
F	598	637	702	81.3	36.7	27.7	94.0	48
G	587	640	726	76.5	36.4	30.3	95.6	60
H	583	647	737	82.3	36.7	27.5	92.0	52
Highly Paraffinic Oil								
I	624	655	700	60.0	35.5	36.7	94.0	—[a]
J	662	694	Cracked	100.0	39.8	32.0	92.0	—[a]
Isoparaffinic Oil								
K	531	566	621	78.2	—	38.0	49.2	—
L	572	604	652	133.0	—	37.0	49.0	—

[a] Viscosity index of oil I = 123.75 and of oil J = 159.27.

of a set of 10 treatments could be taken in a day. A succession of 1 hour each of no light, light, and no light (Wedding *et al.* 1952) was used, with readings at intervals of 30 minutes.

Oil applications were made by means of a vertical spraying tower (ten Houten & Kraak 1949) equipped with a precision nozzle (loaned by the Shell Oil Company). Formulated emulsive spray oil, only, was used in the spraying tower and applied directly to the surface of the leaf in that form. Evidence on file in our laboratory indicates that the effect of petroleum spray oil on the photosynthesis of citrus leaf tissue is due to the oil itself, since results of applications of the emulsive oil directly to the surface of the leaf or in a mixture of an equivalent amount of oil with water were comparable. Accurate control of oil- and air-flow rates into the precision nozzle, and of exposure time, made it possible to apply uniform deposits of oil on both surfaces of the leaf. The original design of the spraying tower was modified so that the shutter between the spray stream and the object was opened by a solenoid controlled by a treadle switch and closed by means of a metal spiral spring. After the adjustments were known for a given predetermined deposit rate, all replicates of a treatment could be sprayed with the selected oil deposit.

Applications of various amounts of oil showed that a deposit of appoximately 600 μg. oil/sq. cm. of leaf surface would produce oil-soaking throughout the entire surface area of the leaf, and this was chosen as the upper limit of the range of oil deposit. Initially, the 10 levels of oil deposits were taken over the full range of 600 μg. with increments of approximately 60 μg. Coefficient of correlation values were low for the regressions calculated with these data, and inspection of the observed coordinate points suggested a difference in slope in the lower and upper parts of the range. Trials indicated better coefficients of correlation for regressions for each of two sections (100 to 300 and 300 to 600 μg.), each with 10 levels at approximately equal increments. Deposits of 100 μg. oil/sq. cm. cause only traces of oil-soaking, and this was taken as the lower limit.

Intervals of 2, 7, and 21 days after application were chosen for determinations of recovery of photosynthesis with time. The choice of three time intervals established the requirement for enough treated leaves for three sets of determinations for each regression of 10 deposit level treatments; so three single-leaf plants were treated with each oil dosage at any given application.

On any given day, therefore, in the course of the experiments, observations of photosynthesis were made for a single regression line of one of the combinations of citrus variety, lower or upper range of oil deposit, and interval of time after application. In each instance preparation of leaf tissue for use in the Warburg apparatus started at 8 A.M.

After application of the oil, the treated plants were put in a glasshouse room with conditions of temperature and light controlled to simulate those of the orchard in late summer. Temperature control permitted a maximum of 92° F. during the day and a minimum of 66° F. at night. During the shorter days of winter, daylight-type fluorescent lights were turned on each day from 4 to 8 A.M. and 3 to 7 P.M. Controls were set for a minimum of 40% relative humidity. As needed, a finely atomized spray of water containing less than 1 p.p.m. of total solids was injected into the air of the upper part of the room.

In general, the equipment and procedures for determinations of photosynthesis were similar to those described by *Wedding* et al. (1952); however, a few changes were made. The water bath was operated at 25° C., the temperature being obtained by either a refrigeration or heating system controlled through a single thermostat. The manometers were not shaken, so we did not use a film of water or any agent to keep the leaf discs in place in the flasks.

Three single-leaf plants were available for each oil-dosage treatment to be used for each of three intervals of time after application. Six leaf discs of 1 sq. cm. each were needed for each flask for each determination. Therefore, for each determination a disc was taken from each side of the midrib of each of the three single-leaf plants. Discs were taken from the base of the leaf to the tip at the $\frac{1}{4}$, $\frac{1}{2}$ and $\frac{3}{4}$ distances, and in each of these positions at approximately halfway between the midrib and the margin. For any given determination each of the three positions from base to tip of leaf was represented twice, randomized by plant and side of leaf.

DISCUSSION OF RESULTS.—The apparent photosynthesis of each given oil treatment was expressed as the per cent inhibition of photosynthesis of the untreated check leaf tissue of the same set of determinations. The observed values obtained seemed to be associated directly with increasing oil deposit in the range of 100 to 300 μg. oil/sq. cm. Data obtained by calculations of regressions of per cent inhibition of photosynthesis on oil deposit are presented in table 2.

Calculated values of mean per cent inhibition of photosynthesis for the selected oil deposit level of 150 μg./sq. cm. vary with different oils and intervals after application; some have a negative sign. The relatively low values of "*r*" and "*F*" in most instances indicated the variability and low order of the response in the 100 to 300-μg. range; however, the amount of inhibiton of photosynthesis *per se* would not necessarily be influenced by significant values of "*r*" and "*F*." In general, the distribution of per cent inhibition of photosynthesis at a depost of 150 μg. oil/sq. cm. appears to occur within the range of 0 ± 10% *i.e.*, no consistent inhibition at the level of 150 μg. Evidence reported by Riehl *et al.* (1958) indicates that an upper limit of 150 μg. oil/sq. cm. would be sufficient for control of the citrus red mite, *Panonychus citri* (McG.), and the California red scale, *Aonidiella aurantii* (Mask.). Therefore, inhibition of photosynthesis by oil spray applied to lemon trees for citrus pest control could be avoided in southern California with proper practices.

It is interesting that the data of table 2 show essentially the same response of Bearss lime and Eureka lemon to inhibition of photosynthesis in the presence of spray oil in the deposit range of 100 to 300 μg./sq. cm. Occurrence of leaf drop following application of oil spray is more likely in the field with lime than with lemon (Ebeling 1950, p. 201).

Since the plants in these laboratory experiments were kept in the glasshouse, they were not exposed to the ultra-violet components of sunlight. Would more inhibition of photosynthesis have occurred among oil-sprayed plants exposed to sunlight containing the natural complement of ultra-violet? Comparable samples of untreated and oil-sprayed leaves were taken from trees of randomized plots established experimentally in orchards near Riverside. Lemons were sprayed with a medium grade, and oranges with a light-medium grade, California proprietary emulsive spray oil used at 1.75% in aqueous mixture and applied manually in thorough coverage in the conventional manner (Carman 1955). The orange trees were sprayed in September and the lemon trees in October. Oil deposits were not determined, but the oil dosage and spray practice were those customarily used for pest control. Data for comparisons of apparent photosynthesis are presented in table 3. The evidence is similar to that of table 2; photosynthesis was not inhibited consistently in the oil-sprayed leaves. Under these conditions the difference in amount of ultra-violet light in the field and in the glasshouse does not appear to be an important factor in the inhibition of photosynthesis of oil-sprayed citrus leaves.

Calculated regression data of percentage of inhibition of photosynthesis of lemon leaf tissue on oil deposit in the range of 300 to 600 μg./sq. cm. are presented in table 4. These data indicate a definite relation between inhibition and increasing oil deposit in the range of 300 to 600 μg. particularly for 2 and 7 days after application. Values of "*r*" are distributed generally around 0.8, and values of "*F*" exceed the requirement for significance. However, the values of "*b*," the coefficient of *X* in the regression equation, are relatively small and imply that the response to small increments of oil would be slight.

Comparisons of the data of table 4 lead to the impression that inhibition of photosynthesis decreases with time after spray application; this seems to be influenced by two

Table 2.—Effects of various petroleum and hydrocarbon oils on the calculated mean per cent inhibition of photosynthesis in leaves at a deposit range of 100 to 300 μg. oil/sq. cm. leaf surface at intervals after application.

		Per Cent Inhibition Photosynthesis		
		μg. Oil/Sq. Cm. Leaf Surface		
		Bearss Lime	Eureka Lemon	
Oil[a]	Days After Application	150	150	300
A	2	3.4± 3.5[b]	—	—
	7	− 3.5± 5.9	—	—
	21	2.5± 2.1	—	—
B	2	—	—	—
	7	11.5± 2.4	—	—
	21	11.2± 5.7	—	—
C	2	6.8± 2.9	—	—
	7	13.1± 5.5	—	—
	21	4.9± 3.9	—	—
D	2	—	—	—
	7	6.5± 4.1	—	—
	21	−11.0± 4.8	—	—
E	2	—	10.1± 1.9	16.2± 3.6
	7	11.0± 5.9	3.8± 4.2	20.3± 4.9
	21	− 5.7± 4.9	14.8± 3.9	18.9± 7.4
F	2	—	12.2± 3.9	20.3± 6.1
	7	9.4± 4.3	− 4.9± 6.4	16.0±10.0
	21	4.2± 6.7	1.5± 5.9	11.4± 9.3
G	2	—	− 3.1± 5.2	− 0.5± 8.7
	7	3.9± 6.1	18.8± 4.6	28.6± 7.7
	21	8.1± 4.4	1.8± 4.4	14.3± 7.4
H	2	—	− 9.5± 4.6	13.2± 7.4
	7	3.3± 6.6	7.0± 5.8	8.8± 9.5
	21	− 5.2± 4.9	4.0± 4.4	− 3.2± 7.2
I	2	5.4± 3.4	2.6± 2.9	35.6± 5.6
	7	0.3± 3.9	− 6.9± 5.2	23.7± 6.1
	21	4.4± 3.3	6.1± 4.8	10.5± 9.0
J	2	0.6± 5.3	5.2± 4.2	13.6± 7.2
	7	5.9± 5.4	− 0.6±14.3	20.5±12.7
	21	− 4.4± 8.1	12.5± 7.1	24.9±12.2
K	2	8.7± 4.5	3.6± 3.9	19.9± 8.0
	7	2.0± 5.2	− 5.5± 4.7	− 8.2± 9.5
	21	7.7± 4.8	3.2± 5.3	6.2±10.8
L	2	10.6± 3.8	6.9± 3.8	30.5± 5.9
	7	8.9± 4.0	1.4± 4.7	23.2± 7.3
	21	13.8±10.4	− 2.1± 3.5	19.0± 5.5

[a] For description of oil, see table 1.
[b] $s_{\bar{y}}$.

Table 3.—Apparent photosynthesis in leaves of citrus orchard trees at various intervals (days) after spray treatment.[a]

Variety and Location	Days After Application	Apparent Photosynthesis, Mean Microliters O_2 per Hour per Flask		L.S.D. at 5%	Per Cent Inhibition
		Untreated Leaves	Oil-Sprayed Leaves		
Lemon					
Orchard A	3	315.9	314.5	28.9	0.4
	7	338.6	336.3	13.6	0.7
	21	338.6	336.2	35.1	0.7
Orchard B	3	286.6	301.7	26.5	− 5.3
	14	289.7	337.0	16.6	−16.3
Navel orange					
Orchard A	3	299.6	350.2	31.6	−22.2
	7	329.0	301.4	36.1	8.4
	21	356.0	343.9	22.7	3.4
Orchard B	3	276.7	249.7	30.8	9.8
	14	301.2	307.5	35.5	− 2.1
Valencia orange					
Orchard A	3	153.9	211.6	61.4	−37.5
	7	308.9	261.7	44.3	15.3
	14	306.7	291.2	50.1	5.1

[a] Trees sprayed with 1.75% proprietary emulsive spray oil in aqueous mixture applied in conventional manner for citrus pest control.

factors: (1) amount of deposit and (2) structural composition of the oil. With the California spray oils E, G, and H, the greatest inhibition occurred 2 days after application; recovery began by the seventh day and was essentially complete at 21 days, as shown by marked decreases in the values of per cent inhibition, "*r*" and "*F*." No good reason is evident for the slower recovery observed for oil *F*. The responses of both inhibition and recovery are more marked at the 500 μg. deposit level than at the 300 μg. deposit level. Inhibition increased from the second to the seventh day at both deposit levels for the highly paraffinic oil J; data for the deposit level of 300 μg. at 21 days may indicate that recovery had started. The viscosity of oil J is higher than that of oil E and its molecular size is larger, as shown by the higher distillation range. As a result, penetration into the leaf tissue may have been slower for oil J and accompanied by an increase in inhibition of photosynthesis from the second to the seventh day after application. For the same reasons dissipation of the oil from the leaf may have been slower and recovery of photosynthesis delayed.

The greatest inhibition of photosynthesis seems to have occurred with the isoparaffinic polyalkylate oil L in the deposit range of 300 to 600 μg. but recovery may have started at 21 days after application. The viscosity of oil L is higher than that of the California spray oils, but it has lower distillation temperatures and is relatively narrow-cut. The oily appearance of leaves sprayed with oil L disappears, and the characteristic pattern of oil-soaking develops in about the same amount of time as that of the California spray oils. These observations indicate that the rate of penetration may have been comparable. The effect on photosynthesis suggests that dissipation of oil L is slower than that of the California spray oils.

As stated earlier, traces of the characteristic dark discoloration of oil-soaking appear along the margin of the leaf within 2 days after application of a deposit of approximately 100 μg. of oil/sq. cm. to both sides of a citrus leaf. As the amount of oil applied is increased, oil-soaking appears laterally along the midrib of the leaf. With further increases of oil deposit the bands of oil-soaking along the margin and midrib widen, shrinking both axes of the unaffected elliptical island between them; a deposit of

Table 4.—Effects of various petroleum and hydrocarbon oils on calculated mean per cent inhibition of photosynthesis of Eureka lemon leaves at a deposit range of 300 to 600 μg. oil/sq. cm. of leaf surface at intervals (days) after application.

Oil[a]	Days After Application	Per Cent Inhibition of Photosynthesis μg. Oil/Sq. Cm. Leaf Surface			Regression Equation $Y=a+bX$[b]	Coefficient Correlation r	F for Slope at 1 and 8 df
		300	500				
E	2	12.8	40.0	=	.136X−28.00	.75	10.50*
	7	5.4	28.8	=	.117X−29.70	.80	14.52**
	21	− 6.6	2.6	=	.046X−20.38	.33	1.01
F	2	15.4	35.8	=	.102X−15.20	.84	19.76**
	7	3.4	36.6	=	.163X−45.50	.81	15.78**
	21	14.7	28.3	=	.068X− 5.66	.76	9.74*
G	2	11.8	49.4	=	.188X−44.60	.97	127.62**
	7	15.0	48.0	=	.165X−34.50	.89	32.03**
	21	10.9	16.1	=	.026X+ 3.10	.45	1.99
H	2	—	—		—	—	—
	7	12.8	33.4	=	.103X−18.10	.84	19.36**
	21	7.1	6.1	=	−.005X+ 8.60	−.06	0.03
J	2	13.1	34.5	=	.107X−18.99	.59	4.36
	7	20.6	44.2	=	.118X−14.80	.80	13.75**
	21	10.2	45.6	=	.177X−42.90	.69	7.95*
L	2	28.1	47.3	=	.096X− .70	.67	6.44*
	7	26.6	45.6	=	.095X− 1.90	.76	4.38
	21	26.0	39.8	=	.069X+ 5.32	.70	7.95*

[a] For description of oil, see table 1.
[b] Y = % inhibition photosynthesis; X = μg. oil/sq. cm. of leaf surface.

approximately 600 μg. causes complete oil-soaking throughout the surface area of the leaf.

The discs of leaf tissue collected for use in the Warburg flasks for determinations of photosynthesis were taken from prescribed positions. Therefore, as increasing increments of oil deposit caused the bands of oil-soaking along the margin and midrib to widen, the respective disc samples of leaf tissue contained an increasing per cent area of oil-soaked tissue. Results of photosynthesis determinations suggested a marked association between inhibition of photosynthesis and percentage of leaf area showing oil soaking. Investigation of the relationship quantitatively appeared desirable.

A definite straight margin between oil-soaked and unaffected tissue was produced in the following manner. Masks of damp filter paper were placed laterally along each side of the midrib and on both the upper and lower side of the leaf, with the position of the edges matched so that a band one-fourth inch wide was left uncovered along each side of the midrib. Oil E was selected and single-leaf Eureka lemon plants were used. The upper side of the leaf was treated with a single application of 650 μg. oil/sq. cm.; the lower side of the leaf was treated with three successive applications of this deposit rate within an interval of 24 hours. This treatment produced a band of oil-soaked tissue 1 cm. wide on each side of the midrib.

Discs of leaf tissue for determinations of photosynthesis were taken across the margin of the leaf between oil-soaked and unaffected tissue, so that $\frac{1}{4}$, $\frac{1}{2}$, $\frac{3}{4}$, and all of the discs, respectively, contained oil-soaked tissue. Discs with none of the area oil-soaked were taken from comparable untreated plants.

Determinations showed that inhibition of photosynthesis increased with the proportion of area oil-soaked, to the level of 50 to 60% inhibition in completely oil-soaked leaf tissue. Plotting of the coordinate points suggested a curvilinear relationship. The independent variable X is percentage of area oil-soaked. Reducing percentages to the decimal equivalent, analyses by means of second-degree polynomial calculations gave the equations: (1) $Y=0.269-0.223X+0.469X^2$, with a multiple correlation coefficient (R^2) of 0.926 for 2 days after application; and (2) $Y=0.176-0.122X+0.510X^2$, with an R^2 of 0.982 for 7 days after application. The linear regressions of percentage of inhibition of photosynthesis on percentage of area oil-soaked for 2 and 7 days after application are, respectively: (1) $Y=12.05+0.362X$ with a correlation coefficient (r) of 0.91 and an F for slope of 33.34 for 1 and 8 degrees of freedom; and (2) $Y=4.01+0.466X$ with an r of 0.824 and an F of 16.89. Inspection of the plotted curves suggested that the relationship $Y=0.55X$ might fit the observed data within the limits of error. The proportion of oil-soaked area of the disc sample of leaf tissue was varied from $\frac{1}{4}$ to $\frac{3}{4}$ as a segment of the surface area of the circle by placing the punch across the margin between oil-soaked and unaffected tissue. Placement of the punch was done visually with the aid of guide marks. The margin of oil-soaked tissue was formed by an expanding front and probably consisted of a gradient. These factors may have contributed materially to the size of the constant for correction of origin derived in the calculated equation given above.

The evidence of Cressman (1941) and of Ebeling (1950, p. 180) suggests that spray oil might also dissipate from tissue showing the discoloration of oil-soaking. In this event, would recovery of photosynthesis take place in oil-soaked tissue? Determinations of photosynthesis were made for oil-soaked citrus leaf tissue comparable to that described above at 2, 7, 10, and 14 days and at intervals of 7 days thereafter for a period of 3 months after application. Inspection of the data indicated that recovery of photosynthesis reached the zone of $0\pm10\%$ inhibition about 9 weeks after the oil was applied. Calculation of the correlation of per cent inhibition of photosynthesis on the $\log_{10}$ of time (X) yielded the equation $Y=1.06692-0.55181X$ with an r value of -0.989 and an F value for slope of 43.719 for 1 and 6 degrees of freedom. Extrapolation to zero inhibition of photosynthesis gives a value of 86 ± 17 days.

Similar correlations of recovery with $\log_{10}$ of time following inhibition after application of oil spray were observed for the functions of translocation of phosphorus (Wedding & Riehl 1958) and of transpiration of citrus (Riehl *et al.* 1958).

Currier & Peoples (1954) used the tetrazolium reaction to distinguish death in *Elodea* treated with various hydrocarbons. Affected cells remained normal green, whereas unaffected cells showed the deep-red formazan color. When citrus leaves were immersed in solutions of either 0.1 or 1.0% 2,3,5-triphenyl tetrazolium chloride in tap water and kept in the dark, color development was patchy and inconsistent. The waxy surface of the leaf may have interfered with penetration of the aqueous solutions.

When a leaf with a cut extending into the blade from the margin was placed in a 1.0% solution, red color developed in a band 1/16 inch wide all along the cut after an exposure of 4 hours in the dark. Navel orange leaves with definite bands of oil-soaked tissue at the margin and midrib were tested in a similar manner, and red color developed all along the edge of the cut across the blade whether the tissue was oil-soaked or not. The leaves were taken 5 weeks after application from trees sprayed in the field on November 12, 1957, with 1.75% medium-grade emulsive oil spray in aqueous mixture.

The test with the tetrazolium reaction demonstrated that the cells in the oil-soaked tissue were not killed by the presence of the spray oil. Inhibition of photosynthesis in oil-soaked tissue seems to be the result of interference with gaseous exchange caused by the presence of the spray oil on or in the tissue. As the oil dissipates, the interference decreases and recovery of photosynthesis occurs.

For the purposes of the following considerations let us represent the margin of a citrus leaf as an arc of a circle. The percentage of total leaf area may then be calculated for bands of various widths. For example, a band of oil-soaked tissue 1 mm. wide around the entire margin of the leaf and on each side of the midrib would include about 12% of the total surface. If the width were increased to 4 mm. throughout, 45% of the surface would be included. Estimation of oil-soaked tissue in the illustration shown by Ebeling (1950, p. 193) for normal deposit indicates that the discolored tissue may not include more than 10% of the total surface area. In a lemon leaf affected to this extent, the principal inhibition of photosnythesis would occur in the oil-soaked tissue and comprise a net of 5 to 6% during the first week of recovery

thereafter; the effect in the remainder of the leaf would be negligible, as shown by the results for the calculated mean per cent inhibition for a deposit of 150 μg. in the regression data of table 2 for the 100 to 300 μg. range.

A succession of 1 hour each of no light, light, and no light was followed in the determinations of photosynthesis with the Warburg apparatus. Readings taken during the periods of darkness measured the respiration. The two sets of respiration values were consistently comparable. No significant differences were found in the respiration of untreated and oil-sprayed leaves. The results for Bearss lime and Eureka lemon agree essentially with those found in earlier work with Eureka lemon (Wedding *et al.* 1952), although some effect might have been expected *a priori* with the relatively high oil deposits or in the oil-soaked tissue.

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The Chemistry and Mode of Action of the Insecticide *O,O*-Diethyl *O-p*-Methylsulfinylphenyl Phosphorothionate and Its Analogues[1]

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ABSTRACT

The insecticide *O,O*-diethyl *O-p*-methylsulfinylphenyl phosphorothionate (Bayer 25141) was studied with regard to its chemical instability to heat, hydrolysis, and oxidation and to the effect of the products formed upon insect toxicity. The compound is readily oxidized to the sulfone and upon heating undergoes internal oxidation-reduction to also form the sulfide. Additionally, the compound apparently isomerizes very readily to form the *S*-ethyl-isomer. The oxidation and isomerization products also form in aqueous systems such as the insect body and as they possess enhanced anticholinesterase activity apparently contribute to the mechanism of toxic action which appears to be that of inhibition of cholinesterase. The bimolecular rate constant for reaction with cholinesterase and the hydroxide-ion catalyzed hydrolysis constant for *p*-substituted methylthio-, methylsulfinyl-, and methylsulfonylphenyl diethyl phosphates and phosphorothionates can be satisfactorily correlated with the electron-withdrawing capacities of the aryl substituents.

The toxicity of organophosphorus insecticides is generally associated with the inhibition of the cholinesterase enzymes. It has been shown that inhibition occurs through a chemical reaction between the enzyme and the organophosphorus inhibitor, and the degree or rate of enzyme inhibition appears to be largely a function of the reactivity of the organophosphorus molecule (Aldridge & Davison 1952, Fukuto & Metcalf 1956). Thus, in a series of diethyl substituted phenyl phosphates, enzyme inhibition rates have been correlated with alkaline hydrolysis rates and other measurements of P—O—phenyl bond reactivity such as Hammett's *sigma* coefficients and shifts in infrared absorption bands.

The insecticide *O,O*-diethyl *O-p*-methylsulfinylphenyl phosphorothionate[2] has recently been shown to be out-

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[2] Bayer 25141, Farbenfabriken-Bayer, Leverkusen, Germany.

standing against certain insect pests. In view of previous work done in this laboratory on the corresponding phosphate analog (Fukuto & Metcalf 1956), it became of interest to investigate the chemistry and mode of action of *O,O*-diethyl *O*-*p*-methyl-thiophenyl phosphorothionate, and its corresponding sulfoxide and sulfone oxidation products and compare these with the phosphate analogs. The insecticidal properties of this series of organophosphorus toxicants are described in a companion paper (Benjamini *et al.* 1959).

MATERIALS AND METHODS.—*Phenols.*—Prepared as previously described were *p*-methylthiophenol, *p*-methylsulfinylphenol, and *p*-methylsulfonylphenol (Fukuto & Metcalf 1956).

Phosphates.—Diethyl *p*-methylthiophenyl phosphate (IV), diethyl *p*-methylsulfinylphenyl phosphate (V), and diethyl *p*-methylsulfonylphenyl phosphate (VI) were prepared as previously described (Fukuto & Metcalf 1956).

Phosphorothionates.—*O,O*-diethyl *O*-*p*-methylsulfinylphenyl phosphorothionate (II) and *O,O*-dimethyl *O*-*p*-methylsulfinylphenyl phosphorothionate (Bayer 25198) both as purified compounds and as P^{32}-labeled radiotracers, were obtained from Farbenfabriken-Bayer through the courtesy of Dr. G. Schrader and Mr. R. J. Geary. The chemical structures of the compounds studied are given in table 1.

O,O-Diethyl O-p-Methylsulfonylphenyl Phosphorothionate (III).—Sodium (1.0 gm.) was dissolved in 16.2 ml. absolute ethyl alcohol. Anhydrous benzene and 7.3 gm. *p*-methylsulfonylphenol were added, and the ethyl alcohol was removed by azeotropic distillation with benzene. The temperature of the mixture was lowered to 70° C. and 8.1 gm. of diethyl phosphorothionochloridate was added with stirring. The mixture was stirred for 4 hours and filtered; the benzene solution was washed, first with dilute sodium hydroxide and then several times with water and dried over anhydrous sodium sulfate. The benzene was removed and the product was distilled in the falling-film molecular still, b.p. 180–200° C. (0.5 mm.), n_D^{26} 1.5265. Most of the product was collected at 195° C. Elemental analysis: per cent calculated for $C_{11}H_{17}O_5PS_2$, C = 40.72, H = 5.28; found, C = 40.76, H = 5.62.

O,O-Diethyl O-p-Methylthiophenyl Phosphorothionate (I).—A mixture of 5 gm. *p*-methylmercaptophenol, 6.8 gm. diethyl phosphorothionochloridate, and 3.8 gm. sodium carbonate in anhydrous acetone was stirred and heated at reflux for several hours. The cooled mixture was filtered, the filtrate concentrated to an oil, taken up in chloroform and washed with 5% sodium hydroxide and water. The solution was dried over sodium sulfate and the product distilled through a falling-film molecular still, b.p. 130° C. (0.1 mm.), n_D^{26} 1.5603. Elemental analysis: per cent calculated for $C_{11}H_{17}O_3PS_2$, C = 45.18, H = 5.85; found, C = 45.54, H = 6.59.

Paper chromatographic separations were accomplished by the reversed phase technic described by March *et al.* (1954), using Whatman No. 1 chromatographic paper impregnated with 50% propylene glycol from absolute ethanol, and developing with a mixture of 7 parts toluene and 3 parts hexane (Skellysolve B). The identities of the various spots were tentatively confirmed by comparison with known compounds, using autoradiography, by appropriate microchemical reactions, by infrared spectrophotometry, and by excising the spots and measuring their anticholinesterase activity. Quantitative evaluation of component spots was accomplished by serial counting of the P^{32} activity (Fukuto & Metcalf 1954).

Table 1.—Properties of compounds studied.

	COMPOUND	I_{50} M	K_e [a] MIN.$^{-1}$	K_{hyd} [b] MIN.$^{-1}$
I	*p*-$CH_3SC_6H_4OP(S)(OEt)_2$	9.2×10^{-6}	3.6×10^{2}	1.4×10^{-2}
II	*p*-$CH_3SOC_6H_4OP(S)(OEt)_2$	7.3×10^{-6}	2.5×10^{3}	4.4×10^{-2}
III	*p*-$CH_3SO_2C_6H_4OP(S)(OEt)_2$	5.2×10^{-7}	9.3×10^{4}	7.3×10^{-2}
IV	*p*-$CH_3SC_6H_4OP(O)(OEt)_2$	3.3×10^{-5}	2.3×10^{3}	3.3×10^{-2}
V	*p*-$CH_3SOC_6H_4OP(O)(OEt)_2$	3.1×10^{-6}	2.4×10^{5}	9.8×10^{-1}
VI	*p*-$CH_3SO_2C_6H_4OP(O)(OEt)_2$	2.5×10^{-7}	1.9×10^{6}	9.3×10^{-1}
VII	*p*-$CH_3SOC_6H_4OP(O)OEt, SEt$	9.8×10^{-8}		
VIII	*p*-$CH_3SO_2C_6H_4OP(O)OEt, SEt$	7.5×10^{-8}		
	p-$O_2NC_6H_4OP(O)(OEt)_2$	2.6×10^{-8}	2.7×10^{7}	1.7

[a] Bimolecular rate constant for reaction with fly cholinesterase.
[b] First order rate constant for hydroxide ion catalyzed hydrolysis.

Anticholinesterase activity (I_{50}) was determined at 37.5° C. by the standard Warburg manometric procedure (Fukuto & Metcalf 1956) using fly head brei. The compounds were incubated with 0.1 *M*. diethyl barbituric acid buffer (pH 9.5) for 24 hours just prior to the anticholinesterase assay, in order to destroy possible traces of highly active pyrophosphates (Aldridge & Davison 1952).

The rate constant K_e for the reaction between the organophosphorus compound and house fly-brain cholinesterase was determined manometrically in double-armed Warburg flasks according to the method described by Aldridge & Davison (1952).

The inhibition of cockroach, *Periplaneta americana* (L.), nerve cord cholinesterase was also determined manometrically. Cockroaches were treated topically with approximately the LD_{50} dose of the compound, the nerve cord removed, washed with water and homogenized in Warburg buffer (2 cords/4 ml. buffer). The homogenate (2.7 ml.) and 0.3 ml. 0.1 *M*. acetylcholine bromide were taken and the degree of inhibition measured in the usual manner.

In vivo experiments with the American cockroach were carried out as follows: Three or 300γ of the P^{32}-labeled compound in 1 microliter of benzene were applied topically to adult cockroaches. At various time intervals the Malpighian tubes, guts, and nerve cords of 10 cockroaches were removed and pooled. Organs were homogenized with 5 ml. of water, and partitioned with equal volumes of chloroform. Aliquots of the water and chloroform phases were counted for total radioactivity and the $CHCl_3/H_2O$ partition ratio was established. Following the application of 300 γ per cockroach the remaining chloroform portion was concentrated and chromatogramed.

In vitro experiments were accomplished by incubating the guts of 10 cockroaches in Krebs Ringer solution (Umbreit *et al.* 1951) with 3 mg. of the P^{32}-labeled *O,O*-diethyl *O*-*p*-methylsulfinylphenyl phosphorothionate in a Warburg manometric flask at 37.5° C. for 4 hours, at the end of which period the entire mixture was homogenized and partitioned with an equal volume of chloroform. The chloroform portion was concentrated and chromatogramed. Controls consisted of an identical run but without the presence of the cockroach gut.

The rates of hydrolysis of the series of compounds under study were determined at 37.7° C. by ultraviolet

Table 2.—Chemical transformation of residue of *O,O*-diethyl *O*-*p*-methylsulfinylphenyl phosphorothionate on surface of cotton leaves.

R_f	Probable Identity[a]	Per Cent Total P^{32} at Indicated Days Following Application					
		0	1	2	4	7	14
0.0	water soluble P^{32}	0.4	5.2	2.9	3.6	1.3	2.3
0.09	VII (*S*-ethyl-II)	1.7	3.5	1.6	3.0	3.6	2.0
0.11	VIII (*S*-ethyl-III)	0.4	1.0	0.6	1.0	0.5	1.7
0.67	II	97.5	90.0	92.0	89.0	92.0	90.0
0.88	III	trace	0.9	1.1	1.8	1.9	3.0
0.98	I	trace	1.3	2.1	0.9	1.1	0.4

[a] See table 1.

Table 3.—Paper chromatographic separation of compounds formed by heating *O,O*-diethyl *O*-*p*-methylsulfinylphenyl phosphorothionate to 140° C. in sealed tube.

R_f	Probable Identity[a]	Per Cent of Total P^{32} on Chromatogram After Indicated Time of Heating				
		0	10 Min.	30 Min.	3 Hr.	7 Hr.
0.0	water soluble P^{32}	2.1	3.9	4.1	8.1	
0.09	VII	0.0	3.8	9.0	16.4	55.0
0.11	VIII	0.0	1.4	2.6	3.6	
0.67	II	95.5	84.0	80.0	47.5	8.0
0.88	III	1.2	2.4	2.8	7.4	9.2
0.98	I	1.3	4.1	2.2	16.6	27.0

[a] See table 1.

spectrophotometry by measuring the change in absorption at 310 mμ for the *p*-methylthiophenyl esters and at 280 mμ for the *p*-methylsulfinylphenyl and *p*-methylsulfonylphenyl esters. Solutions of ester (approximately 1.4×10^{-4} *M.* in ester) were made up in either 0.01 *M.* or 0.2 *M.* sodium hydroxide and the amount of unchanged ester was estimated spectrophotometrically after measured time intervals. A standard curve for each ester was established by plotting absorptions at 310 or 280 mμ against known mole fractions of ester and phenol in standard sodium hydroxide. The rates of hydrolysis all followed first-order kinetics and pseudo first-order constants (K_{hyd}) were calculated in the usual manner.

DISCUSSION OF RESULTS.—*Oxidation and isomerization.*—*O,O*-diethyl *O*-*p*-methylsulfinylphenyl phosphorothionate (II) is a yellowish liquid, b.p. 138–141° C. (0.1 mm.), n_D^{25} 1.540, d^{25} 1.202, which is soluble in most organic solvents and insoluble in water. It is slowly oxidizable in air to form the sulfone *O,O*-diethyl *O*-*p*-methylsulfonylphenyl phosphorothionate, which has recently been patented as an insecticide (Metivier 1957). This was demonstrated by applying a benzene solution of the P^{32}-labeled compound to the surface of cotton leaves and glass plates which were exposed outdoors for 14 days. At intervals, as shown in table 2, portions of the surface residues were separated by paper chromatography and the relative amounts of the components determined.

Isomerization of phosphorothionates to phosphorothiolates is a characteristic phenomenon in which a phosphorothionate rearranges to a phosphorothiolate (Emmett & Jones 1911). This change has a profound effect upon chemical and biochemical properties (Metcalf & March 1953, Fukuto & Metcalf 1954). It was, therefore, of significance to find from paper chromatographic evidence small amounts of the isomeride *O*-ethyl *S*-ethyl *O*-*p*-methylsulfinylphenyl phosphorothiolate (VII) forming on the leaf and glass surfaces along with its sulfone oxidation product *O*-ethyl *S*-ethyl *O*-*p*-methylsulfonylphenyl phosphorothiolate (VIII).

The isomerization process was investigated more critically by sealing 200 mg. P^{32}-labeled II in a small glass capillary and heating it in an oil bath to 140° C., a temperature which rapidly isomerizes parathion and its dimethyl ester (Metcalf & March 1953). Samples were taken after various intervals of heating for paper chromatographic and infrared analysis. The results are presented in table 3 and show the rapid disappearance of the sulfoxide (II) R_f 0.67 and the formation of appreciable amounts of at least four other compounds with different chromatographic properties. The compounds R_f 0.67 and R_f 0.88 were identified as the starting P-S sulfoxide (II) and its oxidation product the P-S sulfone (III) by co-chromatography with known samples and by their relative anticholinesterase activities upon elution and assay at known concentrations as measured by P^{32} activity. (I_{50} values eluted material 2×10^{-6} *M.* and 1.6×10^{-7} *M.*, respectively). The compound R_f 0.98 was identified as the reduction product of the starting material II, P-S sulfide (I) by co-chromatography, anticholinesterase activity (I_{50} eluted material 9×10^{-6} *M.*), and by its reaction with 2,6-dibromo-*N*-chloro-*p*-quinoneimine to form a red dye (Menn *et al.* 1957). This color reaction occurs only with sulfides and was not observed with any of the other compounds studied except the dimethyl analogue *O,O*-dimethyl *O*-*p*-methylthiophenyl phosphorothionate. The presence of both the sulfone and sulfide in the heated sulfoxide indicates an interesting internal oxidation-reduction reaction in which the sulfoxide moiety acts as both oxygen donor and acceptor. A similar reaction of *O,O*-dimethyl *S*-ethyl-2-sulfinylethyl phosphorothiolate has been described by Heath & Vandekar (1957).

The structure of the compound R_f 0.09 was tentatively assigned as the *S*-ethyl isomer of the starting material, *O*-ethyl *S*-ethyl *O*-*p*-methylsulfinylphenyl phosphorothiolate (VII), by its greatly enhanced anticholinesterase activity over the parent compound (I_{50} eluted material 9.8×10^{-8}) and by an indication of the appearance of the P═O bond in infra-red spectrometry. The P═O bond in the *S*-ethyl isomers was found to appear as a small shoulder at 1260–1280 cm.$^{-1}$ rather than as the strong absorption at 1250–1300 cm.$^{-1}$ which is characteristic of the phosphate esters such as para-oxon and diethyl *p*-methylsulfinylphenyl phosphate (V). This was confirmed by spectra from pure model compounds and will be reported in detail elsewhere.

The structure of the compound R_f 0.11 was tentatively assigned as the sulfone oxidation product of VII, *O*-ethyl *S*-ethyl *O*-*p*-methylsulfonylphenyl phosphorothiolate (VIII) by its R_f and enhanced anticholinesterase activity (I_{50} eluted material 7.5×10^{-8} *M.*).

The dimethyl analogue, *O,O*-dimethyl *O*-*p*-methylsulfinylphenyl phosphorothionate was less stable to heat than the diethyl ester. The starting material was present to only 49.0% after 30 minutes. at 140° C., 10.9% after 3 hours and none remained after 7 hours. The total amount of isomerized *S*-methyl ester was 21.5% at 30 minutes and nearly 100% at 7 hours. The corresponding sulfone and sulfide obtained through internal oxidation-reduction of the starting material reached 7.1 and 9.2%, respectively, after 30 minutes, and then declined to 1.7

and 0.7% at 3 hours. None could be found after 7 hours, indicating the greater instability of the dimethyl ester series over the diethyl compounds.

The relative susceptibility of this series of compounds to oxidation and isomerization is of especial interest in connection with the storage life, and persistence of surface residues of insecticide, as well as in the insect body. Both oxidation and isomerization produce compounds of heightened anticholinesterase action (table 1) but also decreased stability, so that the ultimate biological activity represents the net effect of these factors.

Reaction with Cholinesterase.—The reaction of the dialkyl substituted aryl phosphates with cholinesterase is bimolecular and the rate is determined by the magnitude of the induced electrophilic property of the phosphorus atom (Fukuto & Metcalf 1955, Fukuto 1957). A suitable quantitative measure of this effect is provided by Hammett's σ values (Hammett 1940) which measure the electron-withdrawing properties of the aromatic substituents in the meta- and para-positions. For the series under study the respective σ values according to Jaffe (1953) are p-CH_3S −0.047; p-CH_3SO +0.567, and p-CH_3SO_2 +1.049. This would indicate that the successively oxidized states of the p-$CH_3SC_6H_4$-moiety should possess markedly differing rates of combination (K_c) with fly-head cholinesterase. The bimolecular rate constants (K_i) were determined for both the phosphorothionate series I, II, III and the phosphate series IV, V, VI (Table 1), and are plotted along with *O,O*-diethyl *O*-*p*-nitrophenyl phosphate (σ for p-NO_2 = 1.27) against the theoretical values in figure 1. A satisfactory agreement was found for both series with considerably higher velocities being exhibited by the phosphates as would be predicted from the greater electro-negativity of oxygen (3.5) over sulfur (2.5) (Fukuto 1957).

The rates of hydroxide ion catalyzed hydrolysis (K_{hyd}) for this series of phosphorothionates and phosphates were determined as shown in table 1, where the correlation between Hammett's σ constant and hydrolysis rates is apparent. Here again the phosphates exhibit faster hydrolysis rates than the corresponding phosphorothionates.

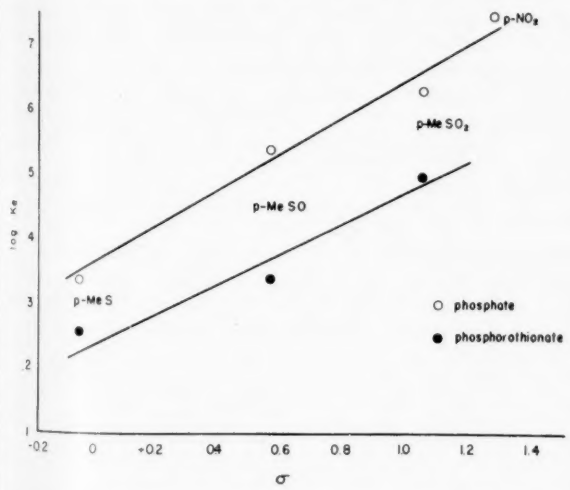

FIG. 1.—Relation of Hammett's sigma constant to bimolecular rate constant K_c for reaction with fly head cholinesterase.

Table 4.—The distribution of P^{32} material in the American cockroach following the topical application of 3 γ and 300 γ of P^{32}-labeled *O,O*-diethyl *O*-*p*-methylsulfinylphenyl phosphorothionate per cockroach.

Hours Following Application	Gut				Nerve Cord		Malpighian Tubules	
	$CHCl_3/H_2O$		γ/gm.		γ/gm.		γ/gm.	
	3 γ	300 γ	3 γ	300 γ	3 γ	300 γ	3 γ	300 γ
1	1.79	—	0.36	—	0.85	—	0.10	—
4	0.61	18.3	0.53	44.5	0.37	3.5	0.65	5.9
9	0.76	—	0.82	—	0.19	—	0.67	—
21	0.45	—	0.68	—	0.073	—	0.85	—

Mode of action.—It is generally agreed that the toxic action of the organophosphorus insecticides is primarily effected by the inhibition of the cholinesterase enzyme of nerve and muscle and the resultant accumulation of acetylcholine at vital centers. The compounds studied here are all *in vitro* anticholinesterases as shown in table 1 and figure 1. When *P. americana* was topically treated with the LD_{90}-LD_{100} of the P-S sulfide I-120γ, the P-S sulfoxide II-10γ, or the P-S sulfone III-10γ, and the ventral nerve cord removed and assayed at the prostrate stage of poisoning, the cholinesterase was found to be 85% inhibited by I, 88% by II and 87% by III, as measured by the Warburg manometric technic.

To determine something of the distribution and metabolism of *O,O*-diethyl *O*-*p*-methylsulfinylphenyl phosphorothionate (II) in *P. americana* the P^{32}-labeled compound was applied topically; and at intervals as shown in table 4, the amounts of P^{32} were determined in the gut, nerve cord and Malpighian tubules. The data indicate that the gut rapidly absorbs the material which in the early stages of poisoning is largely intact esters as shown by the $CHCl_3/H_2O$ partition coefficient and which is subsequently broken down into hydrolytic products. The Malpighian tubules are able to concentrate the P^{32} compounds from the blood as shown by their progresively increasing content of P^{32}. The concentration in the nerve cord was highest after the initial hour and decreased over the period of the experiment suggesting that detoxication and elimination of the compound takes place in the nerve cord, as suggested by Ball & Beck (1951). The relative distribution of P^{32} was of the same general pattern as that found by Fernando *et al.* (1951) after the topical application of P^{32} to *P. americana.*

From the data in tables 1 and 4 it is clear that the quantities of toxicant corresponding to the P^{32} in the central nervous system are more than ample to result in a high level of cholinesterase inhibition. The value of 0.073γ per gm. found after 24 hours is equivalent to about a 2.4×10^{-4} molar concentration which is from 100 to 1000 times that required to produce a high degree of inhibition of the enzyme in the insect central nervous system (table 1), regardless of whether the compound is present in its unmetabolized form or is activated by metabolic oxidation or isomerization.

An indication of the *in vivo* metabolism of P^{32}-labeled II was obtained by applying 300 γ of the compound to *P. americana* and measuring the products present in the gut after 4 hours by paper chromatography. The only compounds which could be identified with certainty, other than hydrolytic breakdown products, were the start-

Table 5.—Paper chromatography of chloroform extract from *in vitro* incubation of P^{32}-labeled *O,O*-diethyl *O-p*-methylsulfinylphenyl phosphorothionate with cockroach gut.

R_f	Probable Identity (Table 1)	Per Cent P^{32}[a]	
		Cockroach Gut	Control
0.00	water soluble P^{32}	3.2	3.2
0.09	VII	3.0	2.0
0.11	VIII	1.2	0.0
0.67	II	87.2	94.5
0.88	III	4.8	0.0
0.98	I	trace	trace

[a] $CHCl_3/H_2O$ partition coefficient total P^{32}—32 for cockroach gut material, 118 for control.

ing material II which comprised 94% of the total chloroform soluble P^{32} and a compound R_f 0.09, probably its isomeride *O*-ethyl *S*-ethyl *O-p*-methylsulfinylphenyl phosphorothiolate (VII) which comprised about 5% of the total. This compound which is 60 times as active as an enzyme inhibitor as its precursor (table 1) must contribute much to the toxicity when liberated *in vivo*, although when applied topically to flies an impure sample of this *S*-ethyl isomeride had an LD_{50} of 10 γ/gm., and was thus only about one-half as toxic as its precursor. This is probably due to lack of stability and increased polar character which limit its contact effectiveness, as is also the case with the parathion *S*-ethyl-isomer (Metcalf & March 1953).

Additional evidence of the metabolism of P^{32} *O,O*-diethyl *O-p*-methylsulfinylphenyl phosphorothionate (II) was obtained by incubating this compound with the cockroach gut *in vitro* and analyzing the metabolites formed by paper chromatography. The data obtained are shown in table 5 and from conclusions previously discussed indicate the formation of appreciable quantities of sulfone (III), *S*-ethyl-isomer (VII) and *S*-ethyl-isomer sulfone (VIII), all powerful anticholinesterases (Table 1, Fig. 1). From these experiments it appears that the metabolism of this compound in the insect follows the same pathways as in the plant (Benjamini *et al.* 1959).

The oxidation of the P=S series to the corresponding P=O analogues as has been demonstrated with parathion (Metcalf & March 1953) and Systox-thiol-isomer (March *et al.* 1955) has not been demonstrated with this series of compounds possibly because of interference from the isomerides which form very readily and interfere with the use of cholinesterase assay and paper chromatography for the detection of the small amounts of diethyl *p*-methylsulfinylphenyl phosphate (V) and diethyl *p*-methylsulfonylphenyl phosphate (VI) which must be presumed to form. It is noteworthy that unpublished studies with P^{32}-parathion applied to *P. americana* have shown that only about 0.1% of the total P^{32} is recoverable as *para*-oxon.

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Contact and Systemic Insecticidal Properties of *O,O*-Diethyl *O-p*-Methylsulfinylphenyl Phosphorothionate and Its Analogues[1]

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ABSTRACT

O,O-diethyl *O-p*-methylsulfinylphenylphosphorothionate (Bayer 25141), its dimethyl analogue, and several reduced and oxidized derivatives were investigated as contact and systemic insecticides. The more active compounds studied were of the same order of contact toxicity as parathion and were also effective systemic insecticides. In plant tissue, the sulfoxides are oxidized to sulfones and also appear to isomerize to *S*-ethyl-isomers.

Previous investigation (Fukuto & Metcalf 1956) showed that the insecticidal potency of substituted phenyl dialkyl phosphates was correlated with substituent groups having strongly electronegative properties and that the *p*-methylthiophenyl (IV—identity as in table 1), *p*-methylsulfinylphenyl (V), and *p*-methylsulfonylphenyl diethyl phosphates (VI) were exceptionally toxic to the house fly, greenhouse thrips, and citrus red mite. It was suggested that the toxicity of IV resulted from its *in vivo* conversion to V and VI. Since it had been shown earlier (Metcalf & March 1953) that parathion and other phosphorothionates were oxidized *in vivo* in insects to the corresponding phosphates, the compound *O,O*-diethyl *O-p*-methylthiophenyl phosphorothionate (I) was prepared in 1955 and shown to be an effective toxicant. The recent availability of its oxidation product *O,O*-diethyl *O-p*-methylsulfinylphenyl phosphorothionate (II)[2] as an experimental insecticide of considerable potential usefulness has prompted this further investigation. Comparative studies have also been made with the dimethyl analogue (VII) *O,O*-dimethyl *O-p*-methylsulfinylphenyl phosphorothionate (Bayer 25198).

MATERIALS AND METHODS.—The structural formulae of the compounds studied are shown in table 1. Compounds I, III, IV, V and VI were synthesized in this laboratory and were purified by alkaline washing and molecular distillation (Fukuto & Metcalf 1956, Benjamini *et al.* 1959). Compounds II and VII were supplied through the courtesy of Dr. G. Schrader and Mr R. J. Geary, Farbenfabriken Bayer, both as highly purified preparations and as P^{32} radiotracers. The materials were tested as freshly prepared w/v acetone solutions using the following experimental procedures.

(1) *Musca domestica* Linn.—One ± 0.05 microliters of the desired concentration were applied topically to 3- to 5-day-old female flies. Mortality data were taken at the end of 24 hours in holding-temperature of 60° F.

(2) *Periplaneta americana* (Linn.).—One to four microliters of the compound in acetone solution were applied topically to the ventral side of the cervical membrane of individual adult cockroaches. LD_{50}'s were taken at the end of 24 hours at 60° F.

(3) *Metatetranychus citri* (McGregor).—Twenty-five adult mites were exposed to each orange which was previously immersed in a standard acetone solution of the compound and air dried. Following a 24-hour exposure period at 70° F., mortality was recorded and the LC_{50}'s were established.

(4) *Tribolium confusum* (Duval).—Beetles were exposed to filter papers 9 cm. in diameter which were previously treated with acetone solutions of the compounds and air dried. The exposure took place in closed petri dishes held at 80° F. for 24 hours.

(5) *Laphygma frugiperda* (J. E. Smith).—Fourth to fifth instar larvae of the fall armyworm were treated topically with 1 to 4 microliters of the acetone solution of the compounds. The material was applied onto the dorsum of the neck. The treated organisms were held at 70° F. and were provided with sweet corn. Mortality was recorded at the end of 10 hours.

(6) *Brevicoryne brassicae* (L.)—Adult cabbage aphids were exposed to cabbage leaves previously treated with acetone solution of the compounds and air dried. Mortality data were taken at the end of a 24-hour exposure in Munger cells at 80° F.

The toxicity to white mice of compound II was deter-

[1] Taken in part from a thesis submitted by E. Benjamini in partial fulfillment of the degree of Doctor of Philosophy, University of California, and supported in part by a research grant from the U. S. Public Health Service.

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[2] Bayer 25141, Farbenfabriken Bayer, Leverkusen, Germany.

Table 1.—Contact toxicity of p-substituted phenyl dialkyl phosphorothionates and phosphates.

	Compound	LD_{50} Micrograms per Gram			LC_{50} Per Cent		
		M. domestica	*P. americana*	*L. frugiperda*	*M. citri*	*T. confusum*	*P. brassicae*
I	*p*-$CH_3SC_6H_4OP(S)(OEt)_2$	11.2	5.2	50.0	0.0013	0.035	0.010
II	*p*-$CH_3SOC_6H_4OP(S)(OEt)_2$	4.5	2.9	7.5	0.0010	0.027	0.0001
III	*p*-$CH_3SO_2C_6H_4OP(S)(OEt)_2$	5.0	3.2	10.0	0.0021	0.031	0.0009
IV	*p*-$CH_3SC_6H_4OP(O)(OEt)_2$	2.0[a]			0.0010		
V	*p*-$CH_3SOC_6H_4OP(O)(OEt)_2$	1.5[a]			0.0008		
VI	*p*-$CH_3SO_2C_6H_4OP(O)(OEt)_2$	2.5[a]			0.0020		
VII	*p*-$CH_3SOC_6H_4OP(S)(OMe)_2$	2.7	5.8	20.0	0.0037	0.038	0.0012
	Parathion	1.0	1.5[b]	2.5			0.0004

[a] Fukuto & Metcalf (1956).
[b] Metcalf & March (1949).

mined following the interperitoneal injection of the compound in 0.1 or 0.05 ml. propylene glycol. Mortality data were taken 24 hours following the injection.

All test animals except for citrus red mites, fall armyworms and cabbage aphids were laboratory reared nonresistant strains. Citrus red mites, fall armyworms and cabbage aphids were collected in fields in southern California which were not previously treated with insecticides.

Systemic toxicity studies were accomplished by pipetting 5 or 10 microliters of the tested compounds onto the base of the stem of 4- to 6-weeks-old cotton plants about 15 cm. above the ground. Treated plants were kept in the greenhouse at 80° F. and were watered once every day. At intervals of 1, 2, 4, 7 and 14 days leaves from similar locations on the treated plants were sampled. Ten wingless adults and last stage nymphs of the cotton aphid, *Aphis gossypii* Glover, were exposed to the leaves in Munger cells for 24 hours at 80° F.

Studies on the translocation and the metabolic fate of compounds II and VII were accomplished as follows: 5 or 10 microliters of the P^{32}-labeled compound were pipetted onto the base of the stem of cotton plants as described previously. The treated plants were kept in the greenhouse at 80° F. At various time intervals an area of approximately 2 to 3 square inches was cut out from each leaf (excluding the cotyledons). The sections from each plant were pooled and 0.5- to 2-gm. portions were homogenized in an all-glass homogenizer with 3 to 5 ml. of distilled water. Part of the slurry was taken for total radioactivity count. The remainder was separated by centrifugation and the aqueous portion was shaken with an equal volume of choroform. The chloroform portion was concentrated almost to dryness under an air stream and the remaining chloroform solution was chromatogramed. The techniques for paper chromatography, quantitative evaluation of P^{32} compounds and identification of the various metabolites are given in a companion paper (Benjamini *et al.* 1959).

DISCUSSION OF RESULTS.—*Contact toxicity.*—The comparative contact toxicities of the compounds discussed to several species of insects is given in table 1. To *Musca domenstica* the phosphates were more toxic than the phosphorothionates which is in agreement with the results obtained with para-oxon and parathion and with most comparative information available (Metcalf 1955). The order of toxicity for both series varies with the *para*-substituent and is in general: $CH_3S < CH_3SO_2 < CH_3SO$. However, as described elsewhere (Benjamini *et al.* 1959), the order of effectiveness in the inhibition of flyhead cholinesterase is $CH_3S < CH_3SO < CH_3SO_2$, and since it is believed that the toxicity of these compounds to insects depends primarily upon their ability to act as irreversible inhibitors of this enzyme (Fukuto & Metcalf 1956, Benjamini *et al.* 1959) it must be concluded that the sulfones III and VI are less effective in the penetration of the insect nerve sheath and perhaps are also too susceptible to hydrolytic degradation to reach an optimum concentration at the site of action.

In the evaluation of the phosphorothionates against the other test species, compound II with the *p*-methylsulfinyl substituent was the most consistently toxic of the thiono-analogues and was of the same order of toxicity as parathion (table 1). The LD_{50} by intraperitoneal injection II to the white mouse was 20–25 mg. per kg. as compared with parathion at 20–24 mg. per kg.

Systemic toxicity.—The systemic toxicity to *Aphis gossypii* feeding on cotton leaves of plants topically treated at the base of the stem with compounds I-VII is shown in table 2. For the diethyl esters the activity was in order I<III<II<VI<V<IV. The information in table 2 clearly indicates that the phosphates are better systemics than the corresponding phosphorothionates, as might be expected from the greater water solubility of the former. The sulfones III and VI are apparently too polar to penetrate the plant cuticle and may be sufficiently unstable so that they hydrolyze rapidly in the plant to inactive products. They were less systemic than the sulfides I and IV which were approximately as effective as the sulfoxides II and V.

Rates of absorption and translocation.—Since it was indicated from table 2 that the *O,O*-diethyl *O*-*p*-methylsulfinylphenyl phosphorothionate (II) was appreciably systemic, it was of interest to compare the rate of absorption and translocation of this compound and that of its dimethyl analogue (VII) with a standard systemic insecticide, *O,O*-diethyl *S*-(2-ethylthio) ethyl phosphorodithioate (Bayer 19639) or Di-Syston[3] (Metcalf *et al.* 1957). This was accomplished by means of P^{32}-labeled radioisotopes of the three compounds. The rates of accumulation of P^{32} (calculated as the respective compounds) in cotton leaves following application of 10 microliters (λ) to the stem is shown in table 3. Plots of these values against time give roughly linear relationships and when the slopes of these lines were compared it was determined that the relative rates of translocation were diethyl ester (II) 1.0, dimethyl ester (VII) 2.0, and Bayer 19639, 6.3.

Plant Metabolism.—The rates of hydrolytic decomposition of systemic insecticides can be measured by the

[3] Trademark Chemagro Corporation, New York.

Table 2.—Systemic toxicity of stem applications on young cotton plants to *Aphis gossypii*.

	Compound[a]	Dosage (Microliters per Plant)	24-Hour Per Cent Mortality After Days Shown				
			1	2	4	7	14
I	PS-sulfide	5	3	0	7	33	5
		10	5	10	10	35	
II	PS-sulfoxide	5	10	3	0	30	45
		10	10	25	50	90	
III	PS-sulfone	5	7	0	3	10	0
		10	5	20	30	75	
IV	PO-sulfide	5	50	90	100	100	75
		10	65	85	100	100	
V	PO-sulfoxide	5	5	93	73	86	85
		10	25	65	65	100	
VI	PO-sulfone	5	10	30	10	66	15
		10	15	85	100	100	
VII	Dimethyl PS-sulfoxide	5	0	87	67	27	20
		10	55	70	100	100	

[a] See table 1.

Table 3.—**Rates of translocation and degradation of *O,O*-diethyl *O*-*p*-methylsulfinylphenyl phosphorothionate, *O,O*-dimethyl *O*-*p*-methylsulfinylphenyl phosphorothionate, and Bayer 19639 in cotton plants following topical application of 10λ to the stem.**

Days Following Application	P.p.m. in Leaves from P^{32}					
	Bayer 19639		*p*-$CH_3SOC_6H_4OP(S)(OEt)_2$		*p*-$CH_3SOC_6H_4OP(S)(OMe)_2$	
	Total	Hydrolyzed	Total	Hydrolyzed	Total	Hydrolyzed
1	12.8	2.4	4.0	0.8	8.4	2.1
2	23.4	3.4	19.2	2.8	15.2	5.9
4	110.0	14.2	34.2	6.6	63.0	28.0
7	201.0	50.0	44.0	8.3	88.0	38.7
14	603.0	168.0	80.0	31.2	162.0	121.0

$CHCl_3/H_2O$ partition coefficients of the P^{32}-labeled material in the leaves. The intact esters partition about 99:1 in favor of chloroform, while the partially hydrolyzed dialkyl phosphoric acids partition about 99:1 in favor of water. The residues of translocated material in cotton leaves shown in table 3 have been corrected for chloroform partitioning material and the values show that the approximate relative rates of decomposition are: Bayer 19639, 1.0, *O,O*-diethyl *O*-*p*-methylsulfinyl phenyl phosphorothionate (II) 1.4, and *O,O*-dimethyl *O*-*p*-methylsulfinylphenyl phosphorothionate (VII) 2.7. The approximately two times more rapid hydrolysis of the dimethyl ester (VII) over the diethyl ester (II) is in accord with the relative rates of hydroxide ion catalyzed hydrolysis of the dialkyl *p*-nitro-phenyl phosphorothionates where the dimethyl ester hydrolyzed 3.0 times as fast as the diethyl ester, parathion (Metcalf & March 1953b).

Paper chromatography was applied to the identification of the chloroform soluble plant metabolites found in cotton leaves after application of P^{32}-labeled *O,O*-diethyl *S*-*p*-methylsulfinylphenyl phosphorothionate (II) to the plant stems. Six major spots were found on the developed chromatograms as indicated in table 4. Their identities were tentatively determined as described by Benjamini *et al.* (1959) from comparisons with standard substances and by appropriate microchemical and biochemical tests. The spot at R_f 0.0 is hydrolyzed water soluble P^{32} carried over in the extraction process. The compound R_f 0.09 is apparently the isomeride *O*-ethyl *S*-ethyl *O*-*p*-methylsulfinylphenyl phosphorothiolate and is the principal plant metabolite. The compound R_f 0.11 appears to be its sulfone oxidation product *O*-ethyl *S*-ethyl *O*-*p*-methylsulfonylphenyl phosphorothiolate. Compound R_f 0.67 is clearly the parent compound (II), and the compound R_f 0.88 is its sulfone oxidation product *O,O*-diethyl *O*-*p*-methylsulfonylphenyl phosphorothionate (III). The trace material R_f 0.98 is apparently the sulfide *O,O*-diethyl *O*-*p*-methylthiophenyl phosphorothionate (I) formed by reduction (Benjamini *et al.* 1959).

To determine if the chemical transformations detected

Table 4.—**Paper chromatographic separation of chloroform soluble metabolites in cotton leaves following application of P^{32} *O,O*-diethyl *O*-*p*-methylsulfinylphenyl phosphorothionate to stem.**

R_f	Probable Identity (Table 1)	Per Cent P^{32} in Chloroform Extract After Days Shown				
		1	2	4	7	14
0.0	water soluble P^{32}	7.4	1.7	10.8	5.6	15.9
0.09	*S*-ethyl II	15.0	13.2	15.0	19.8	23.1
0.11	*S*-ethyl III	0.0	9.2	10.0	4.2	6.0
0.67	II	77.0	74.0	58.0	67.0	50.0
0.88	III	0.5	2.0	4.3	2.9	5.8
0.98	I	trace	trace	1.2	0.5	trace

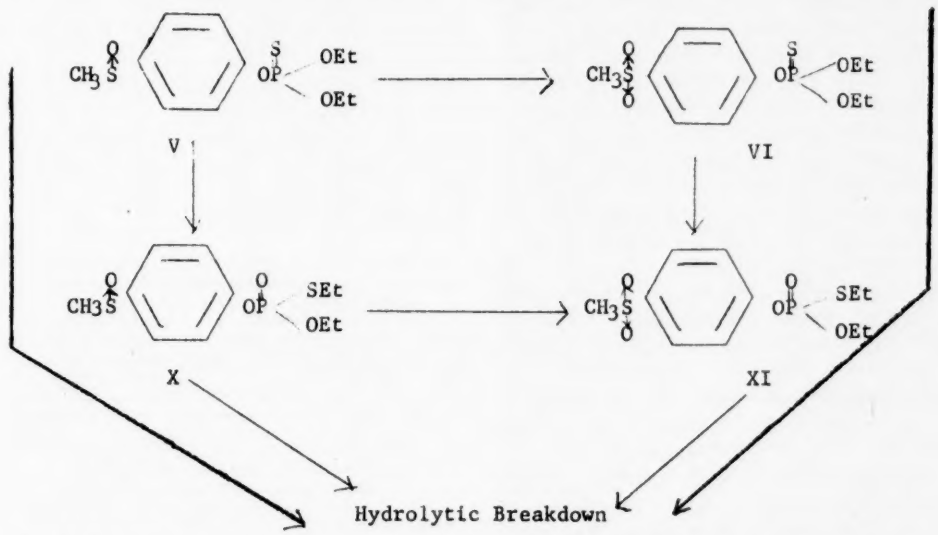

FIG. 1.—Proposed metabolic pathways for *O,O*-diethyl *O*-*p*-methylsulfinylphenyl phosphorothionate in cotton leaves.

in the P^{32} material from the leaf interior were also produced in surface residues, a freshly prepared benzene solution of the sulfoxide (II) was painted upon the cotton leaves and glass plates which were exposed to light and air (Benjamini *et al.* 1959). After 14 days no significant amount of the *S*-ethyl isomeride had appeared, although small amounts of sulfone (III) were found on both surfaces as a result of atmospheric oxidation. These data suggest that both the oxidation and isomerization processes occur chiefly within the leaf interior where they are catalyzed enzymatically and/or hydrolytically. A suggested scheme for the plant metabolism of the sulfoxide (II) is presented in figure 1.

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Thiodan Residues on Corn Plants[1,2]

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ABSTRACT

The persistence of Thiodan residues was studied on corn plants treated for control of the European corn borer, *Pyrausta nubilalis* (Hbn.), with a water emulsion formulation of Thiodan at a rate of 1.5 lbs. of Thiodan per acre and a 5% granulated formulation of Thiodan at a rate of 1.0 lb. of Thiodan per acre. Samples of corn were collected during a 9-week period after treatment; these samples were chopped, extracted, and the extracts analyzed for Thiodan by three analytical methods: titrimetric organic chlorine, colorimetric sulfur dioxide evolution, and house fly bioassay. The initial Thiodan residues on the emulsion-treated corn were much higher than on the granular-treated corn. However, from about 1 week after treatment until the experiment was terminated, only slight differences were found in the Thiodan residues resulting from the two treatments. Residues of Thiodan from the granulated formulation persisted on the corn plants as long as or longer than residues from the emulsion formulation. Small but detectable residues persisted longer than 9 weeks after application of both insecticidal formulations.

It has been reported that Thiodan (6,7,8,9,10,10-hexachloro-1,5,5*a*,6,9,9*a*-hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3-oxide) has shown promise in controlling a large number of insect species (Finkenbrink 1956). Cowan *et al.* (1957) reported that Thiodan showed promise in controlling the boll weevil, *Anthonomus grandis* Boh., and the bollworm, *Heliothis zea* (Boddie). Thiodan also has been effective in controlling the cabbage looper, *Trichoplusia ni* (Hbn.) on cotton (Parencia *et al.* 1957) and the cyclamen mite, *Steneotarsonemus pallidus* (Banks), on strawberries (Allen *et al.* 1957). Roussel & Clower (1957) reported that Thiodan was ineffective in controlling a chlorinated hydrocarbon insecticide-resistant strain of the boll weevil. Guyer *et al.* (1957) reported poor control of the maggot complex on spinach with Thiodan. Thiodan has shown some promise in controlling the European corn borer, *Pyrausta nubilalis* (Hbn.) (Fairchild 1957).

The use of granulated insecticides for the control of the European corn borer is a recent development. Cox *et al.* (1956a, 1956b) reported that granulated insecticide formulations are equal to or somewhat better than water emulsion formulations for controlling the European corn borer. Fahey (1957) stated that corn plants, *Zea mays*, treated with DDT granules had less DDT residue immediately after treatment, at ensilage time, and at stover time, than plants treated with either a water emulsion of wettable powder supension of DDT.

The work reported herein is the concluding portion of several investigations involving Thiodan. A previous paper (Lindquist & Dahm 1957) summarized some chemical and biological experiments with Thiodan. The residue dissipation experiments reported here were designed to: (1) compare the dissipation of Thiodan residues on corn plants treated with Thiodan water emulsion and granulated formulations and (2) evaluate three methods of analyzing Thiodan residues.

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MATERIALS AND METHODS.—Field corn grown on the Iowa State College Experimental farm near Ankeny, Iowa was used in these studies. The corn was 3 to 4 feet tall when treated. The methods of applying the Thiodan formulations to the corn were the same as those used for first brood, European corn borer control at the state and federal cooperative European Corn Borer Laboratory near Ankeny, Iowa.

The Thiodan emulsion formation was applied at a rate of 1.5 lb. of Thiodan per acre with a spray volume of 10 gallons per acre at a pressure of 40 lb. per square inch. A 5% granulated formulation of Thiodan was applied at a rate of 20 lb. per acre; this was equivalent to 1.0 lb. of actual Thiodan per acre. Each treatment was applied to one block of corn plants; each block consisted of 16 adjoining 100-ft. rows of corn. Each treatment block was divided into four, 4-row plots. The emulsion formulation was applied June 26, 1957 and samples for residue analysis were collected 1, 9, 20, 34, and 65 days after application. The granulated formulation was applied June 29, 1957 and samples were collected 1, 6, 17, 31, and 62 days after application.

The sampling procedure consisted of gathering a 10-plant sample from the middle two rows of each plot; the first plant was taken three to four paces into the row. Succeeding plants were taken at three-pace intervals, alternating between the two rows. Two 10-plant samples were taken for controls on each sampling date from unsprayed corn in the same growing area. All plants were cut about 2 inches above the ground. Each 10-plant sample was cut into approximately 2-inch pieces with a small feed chopper; the resulting sample was mixed thoroughly before sampling. To extract the insecticidal residues, a 400- or 500-gm. sample was placed in a wide-mouth, 1-gallon jar with either 2 or 2.5 ml. of solvent per gram of plant material. The jars were rolled mechanically on a machine described by Batchelder & Berndt (1950) for 1 hour to effect extraction. The samples collected 1 day after treatment and on July 5 (9 and 6 days after application of emulsion and granulated formulations, respectively) were extracted with Skellysolve B; the remaining samples were extracted with a solvent mixture consisting of 15% diethyl ether and 85% Skellysolve B (v/v). The extracts were washed twice with 75 ml. of distilled water to remove inorganic chlorides and were stored in glass bottles in the dark until the analyses were run. Two composite samples of chopped corn from each sampling date were dried to determine moisture content of the plants; all residue analysis values were corrected to 90% water content in the corn plant samples for comparison.

Three analytical procedures were used to estimate Thiodan residues: (1) a titrimetric organic chlorine method, (2) a colorimetric method based on determination of sulfur dioxide evolved from Thiodan and (3) a biological assay method employing the house fly, *Musca domestica* L. The degree of correlation between the residue data obtained by the three methods would indicate not only to what extent the technical Thiodan originally applied was present in the residue samples but also whether the Thiodan had been altered under field conditions. All calculations of residues were based on the Thiodan molecule. Duplicate organic chlorine analyses and single sulfur dioxide and biological assays were performed with portions of each extract. The results of the three analytical methods could be compared directly with each other because the three types of assays were performed with aliquots of the same residue extract.

The titrimetric organic chlorine analyses were made according to the method developed by Phillips & De-Benedictis (1954) as modified by Rusk (1957). This method is based on the reduction of chlorinated organic compounds with metallic sodium to liberate chloride ions; the latter are determined by titrating with a standard silver nitrate solution.

The colorimetric sulfur dioxide method was developed by Jones (1957). Sulfur dioxide is evolved from Thiodan by acid hydrolysis using a method similar to that proposed by Watson (1956) for Aramite. The evolved sulfur dioxide is absorbed and determined by a modification of the methods of Paulus *et al.* (1954) and Urone & Boggs (1951). The absorbing solution contains glycerol and sodium hydroxide; the action of acid-bleached, basic fuchsin-formaldehyde on the sulfur dioxide in this solution produces a purple color which is proportional (within a limited range) to the amount of sulfur dioxide present. Extracts of corn samples from the last two sampling dates were fortified with known amounts of technical Thiodan because of the small amounts of Thiodan present. These analytical data were corrected by subtracting the known amounts of added Thiodan.

The biological assay technique used for estimating Thiodan residues was essentially the same as that used by Pfaeffle (1958) for estimating Guthion residues on alfalfa. An indirect method of assay was employed in which the magnitude of the reaction caused by a fixed dose of the insecticide was the dependent variable (Bliss 1956). Burn *et al.* (1950) state that if the test preparation is truly a dilution or concentration of the standard, the two dose-response regression lines must be parallel, assuming that the relationship of response versus log-dose is linear. In these experiments a six-point assay was employed; each standard and test assay consisted of three dose levels so chosen that the resulting mortalities of the test insects were expected to fall between 15 and 85%. An insecticide-susceptible, laboratory strain of house flies was used to provide the 3- to 4-day old female flies used in the bioassays; 50 flies were used per dose in both test and standard assays. All the flies used for one assay series were the same age and were taken from the same holding cage. The flies were reared according to the procedure specified by the Chemical Specialties Manufacturers Association for the Peet-Grady method of testing fly sprays (Anonymous 1958). The test chambers consisted of 1-quart, wide mouth Mason jars fitted with a screw cap and wire screen to confine the flies.

A measured volume of either a standard solution of Thiodan (standard preparation) or a plant extract (test preparation) was added to each jar. To conserve parallelism between the standard and test assays, the same amount of plant extract was present at each dose level of the standard and test assays. This was done by adding the same total amount of corn plant extract (test or control extracts by themselves or a combination of these two extracts) to all three dose levels of the test assays and the corresponding levels of the standard assay. A typical standard assay series consisted of the following

quantities of Thiodan and control corn extract: 20, 30, and 40 μg. of Thiodan plus 15 ml. of control extract for each dose. The corresponding test assay series consisted of 5, 10, and 15 ml. of test extract (from corn samples gathered 9 days after treatment with the emulsifiable concentrate formulation described above) plus 10, 5, and 0 ml. of control extract, respectively. The extracts of the last three sets of corn samples collected were fortified with technical Thiodan because of the small amounts of residue present. These analyses were corrected for the added Thiodan by subtraction.

After the appropriate solutions and extracts were added to the Mason jars, 0.2 mg. of corn oil (Mazola) was added to each jar to achieve an even distribution of the toxicant and to make the toxicant more easily picked up by the flies as they walked and rested on the inner surface of the jar. The jars were then rolled mechanically (Batchelder & Berndt 1950) in a horizontal position to evaporate the solvent and distribute the toxicant on the inner walls of the jars. Fifty female flies were placed in each jar; the flies had been previously anesthetized with carbon dioxide for sexing and counting. The flies were held in the treated jars for 24 hours at 80 ± 2° F. after which the number of dead and moribund flies were counted. The flies were fed a mixture of sugar and water during the exposure period. The number of moribund flies was included with the dead flies in the statistical analysis of the bioassay data. When necessary, a correction for mortality among the control flies was calculated using Abbott's formula (Abbott 1925). The bioassay data were analyzed by Berkson's minimum logit chi-square method (Berkson 1953). The statistical analyses included a test for linearity of all assays and a test for parallelism between the test assays and standard assay of a series. In addition to the assays described above, biological assays were also performed to determine the toxicity of Thiodan alcohol (1,4,5,6,7,7-hexachloro-2,3-bis(hydroxy methyl)-bicyclo [2.2.1] heptene-5), and to determine whether Thiodan alcohol had any effect on the toxicity of Thiodan.

Experiments were performed with chopped corn to determine the per cent recovery of added known amounts of Thiodan (added as technical Thiodan and as a granulated formulation of Thiodan) and Thiodan alcohol. The samples were extracted with either Skellysolve B or the 15% diethyl ether-85% Skellysolve B mitxure. Thiodan alcohol was included because of the possibility that it could be a decomposition product of Thiodan. A study also was made of the recovery of these materials from frozen and thawed corn samples using the 15% diethyl ether-85% Skellysolve B mixture as the extraction solvent. The technical Thiodan and Thiodan alcohol were added to the corn samples as acetone solutions and the acetone evaporated before extracting or freezing the samples. A weighed amount of the granulated Thiodan formulation was added to the corn samples. The samples were extracted with 2 ml. of the diethyl ether-Skellysolve B mixture per gram of plant material. The fresh corn samples were extracted by rolling for 1 hour and the frozen and thawed samples were rolled for 2 hours.

RESULTS AND DISCUSSION.—This study of the dissipation of Thiodan residues on corn plants, following application of water emulsion and granulated formulations for first brood European corn borer control, indicated that small but detectable residues persisted longer than 9 weeks after application of the insecticide (table 1). The actual residues remaining 1 day after application of the Thiodan water emulsion formulation were almost 12 times greater than those remaining 1 day after applying the granulated formulation of Thiodan. However, if one considers the poor recovery of the granulated Thiodan formulation from corn samples extracted with Skellysolve B (26% in table 2 and see also discussion below) and the 1.5 times higher rate of Thiodan application in the case of the emulsion formulation, the residues remaining on the emulsion-treated corn, 1 day after treatment, were only about two times greater than those remaining on the granular-treated corn the same interval after application. Approximately 1 week after application of the two formulations of Thiodan, there were no appreciable differences in residues between the two treatments. The residue data obtained by the three analytical methods are quite similar. On the last two sampling dates (July 30 and August 30, table 1) the sulfur dioxide evolution method of analysis and the bioassays showed consistently lower Thiodan residues than the organic chlorine analyses. This difference suggested that the residues obtained by the organic chlorine method of analysis included, in addition to Thiodan, one or more organic compounds which contained chlorine, were nontoxic to house flies, and probably did not contain the sulfur moiety of the Thiodan molecule.

The lower initial Thiodan residues resulting from application of the granulated formulation seems to parallel the findings of Cox *et al.* (1956a) with DDT emulsion and granulated formulations. In these experiments with Thiodan, the lower residues from the granulated formulation could also be due to: the lower application rate of Thiodan in the granulated formulation (*i.e.* 1.0 lb. Thiodan per acre versus 1.5 lb. of Thiodan per acre in the emulsion application), variations in application equipment, and the difficulty in recovering Thiodan with Skellysolve B (see table 2). The persistence of the Thiodan residues on corn plants treated with the granulated formulation probably can be explained by the assumption that the granules rolled into the whorls and leaf sheaths of the corn plants (*c.f.* the work of Fahey *et al.* (1956) with DDT emultions and granules), where Thiodan was protected from the direct action of the sun, wind, and rain. On the other hand, the Thiodan residues from the emulsion treatment were located mostly on the corn leaves where the climatic elements could deteriorate and remove the toxicant.

Regarding the analytical procedures employed, the organic chlorine method of analysis is not specific for Thiodan because most organic chlorine-containing insecticides will give a positive test. The sulfur dioxide evolution method of analysis is believe to be more specific than the organic chlorine method for the analysis of Thiodan residues because it is known that Thiodan readily undergoes hydrolysis with loss of sulfur dioxide (Lindquist & Dahm 1957). Furthermore, Thiodan and Thiodan alcohol, the latter compound being a possible decomposition product of Thiodan, can be differentiated by the positive response of Thiodan to the sulfur dioxide evolution method of anlysis. The house fly bioassay method of residue analysis estimates the total toxicity of the compounds in the plant extracts and also provides a means of

Table 1.—Summary of dissipation of Thiodan residues, with standard deviations, on corn treated with a water emulsion or a 5% granulated formulation.[a]

	Emulsion (thiodan, 1.5 lb./A.)				Granules (thiodan, 1.0 lb./A.)			
Date Sampled (1957)	Days After Treatment	Organic[b] Chlorine (p.p.m.)	Sulfur[c] Dioxide (p.p.m.)	Bioassay[c] (p.p.m.)	Days After Treatment	Organic[b] Chlorine (p.p.m.)	Sulfur[c] Dioxide (p.p.m.)	Bioassay[c] (p.p.m.)
June 27	1	91 ±16	104 ±12	110 ±33	—	—	—	—
June 30	—	—	—	—	1	8.7±2.7	9.1±2.9	8.8±3.6
July 5	9	5.2± 0.8	4.1± 0.5	5.4± 0.9	6	4.3±0.5	4.4±0.2	3.9±1.3
July 16	20	2.2± 0.5	0.7± 0.2	1.9± 1.0[e]	17	2.5±0.4	1.5±0.6	1.5±1.8[e]
July 30	34	1.3± 0.2	0.3± 0.3[d]	0.0± 0.3[e]	31	2.2±0.2	0.2±0.2[d]	0.0±1.0[e]
Aug. 30	65	2.7± 0.6	0.0± 0.2[d]	0.1± 0.6[e]	62	1.8±0.5	0.1±0.2[d]	0.5±0.4[e]

[a] Residues were estimated by three analytical methods: organic chlorine, sulfur dioxide evolution, and house fly bioassay. All residue analysis values have been corrected to 90% water content in the corn plant samples.
[b] Each value represents the average of eight analyses; *i.e.*, two analyses per plot per sampling date. These analysis values are reported in terms of Thiodan.
[c] Each value represents the average of four analyses; *i.e.*, one analysis per plot per sampling date.
[d] These extracts were fortified with 2 μg. of technical Thiodan per ml. of extract; the analysis values were corrected by subtraction.
[e] These extracts were fortified with 4 μg. of technical Thiodan per ml. of extract; the analysis values were corrected by subtraction.

distinguishing between Thiodan and Thiodan alcohol since small amounts of the latter compound are not toxic to house flies. For example, doses of 10, 50, 100 and 200 μg. of Thiodan alcohol per bioassay test jar resulted in 24-hour mortalities of 0, 0, 0, and 12%, respectively, of the 50 female flies per jar. Furthermore, Thiodan alcohol appeared to have no effect on the toxicity of Thiodan to female house flies used in these bioassays when the ratio of Thiodan alcohol to Thiodan was 1:1 and 2:1.

The recoveries of added known amounts of Thiodan (as technical Thiodan and as a granulated formulation of Thiodan) and Thiodan alcohol from chopped corn, using Skellysolve B or a mixture of 15% diethyl ether and 85% Skellysolve B (v/v) as the extracting solvents, are shown in table 2. Also shown are recoveries of the same materials from frozen and thawed samples using the diethyl ether-Skellysolve B mixture. Skellysolve B is not a good solvent for extracting Thiodan alcohol or Thiodan added in the granular formulation to fresh corn samples. The diethyl ether-Skellysolve B mixture extracted considerably more of the added Thiodan and slightly more of the added Thiodan alcohol from fresh corn samples than from frozen or thawed samples. The lower recoveries of Thiodan and Thiodan alcohol from the frozen and thawed samples probably were due to the presence of water on the outer surface of the plant parts. This water came from inside the plant cells which had ruptured upon being frozen, and probably prevented the solvent from coming into intimate contact with the surface of the chopped plant parts. By comparison, the surface of the fresh corn samples was nearly dry. The fact that Skellysolve B was a poor extraction solvent for Thiodan in a granulated formulation and Thiodan alcohol was not discovered until after the samples collected 1 day after treatment and on July 5 had been extracted with Skellysolve B. All subsequent samples were extracted with the diethyl ether-Skellysolve B mixture.

Table 2.—Mean per cent recoveries of added known amounts of Thiodan (as technical Thiodan and as a granulated formulation of Thiodan) and Thiodan alcohol from fresh, frozen, and thawed corn samples.[a]

Condition of Corn When Extracted	Extraction Solvent	Per Cent Recoveries[b] Technical Thiodan	Thiodan Granules	Thiodan Alcohol
Fresh	SKB[c]	91 (1)	26 (2)	47 (1)
	DE+SKB[d]	96 (1)	105 (2)	94 (2)
Frozen	DE+SKB	80 (1)	86 (2)	92 (2)
Thawed	SKB	67 (1)	—	—
	DE+SKB	73 (1)	86 (2)	92 (2)

[a] Extracted with either Skellysolve B or a mixture of 15% diethyl ether and 85% Skellysolve B (v/v). A ratio of 2 ml. of solvent per gm. of plant material was used. The fresh samples were extracted for 1 hour and all others for 2 hours; from three to seven organic chlorine analyses per extract were used to estimate per cent recoveries.
[b] 20 to 50 p.p.m. of technical Thiodan added in an acetone solution; 5 to 15 p.p.m. of Thiodan added as a 5% Thiodan granulated formulation; 10 to 40 p.p.m. of Thiodan alcohol added in an acetone solution. Number of samples extracted given in parentheses.
[c] SKB = Skellysolve B.
[d] DE+SKB = 15% diethyl ether and 85% Skellysolve B (v/v).

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Response of the Pea Aphid to Chemical Repellents[1]

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ABSTRACT

During 1956–57 a series of tests were conducted with methyl pentanone, camphene, 10-undecenoic acid and paradichlorobenzene against the pea aphid (*Macrosiphum pisi* (Harris)) in an olfactometer and in the field to evaluate the effect of some of the factors determining the degree of repellency. The factors determined were the effect of concentration, the presence or absence of winged forms, laboratory-reared versus field-collected, number of test cages and the length of repellency of treated surfaces. Results with the different test materials varied, but concentration and the length of exposure of a treated surface to an air stream had the greatest effect on the index of repellency. All of the materials tested resulted in fair or good repellency of pea aphids in laboratory tests. In field tests no repellency was experienced. It was concluded that since no material was repellent for more than 4½ hours in the laboratory, and field counts were not made for 24 hours after application that any initial repellency had disappeared before field counts were made.

Paradichlorobenzene was more repellent toward the winged form of *M. pisi* than the wingless form, while camphene and methyl pentanone were slightly more repellent to the wingless forms. Ten-undecenoic acid exhibited no important differences between the strains. Very little difference existed between the response of laboratory-reared and field-collected insects to the repellents, indicating that differences in vitality did not account for differences between laboratory and field tests.

The olfactometer has shown that a number of chemical substances are repellent to a variety of agriculturally important insects (Chamberlain 1956). It remained to be determined whether a substance found repellent in laboratory tests with the olfactometer was repellent to the same insects in field tests. Before conclusions can be made on the results of field test versus laboratory tests it is important to determine if any differences exist between the laboratory test insect or conditions and those found in the field, and, how differences in these factors may affect the results of an experiment.

The purpose of this paper is to report the results of tests conducted on a single insect, the pea aphid, *Macrosiphum pisi* (Harris), with repellent substances both in the laboratory and in field tests. Such factors as winged or wingless condition, or laboratory-reared or field-collected populations may have a decided bearing on laboratory and field comparisons. These factors were evaluated in laboratory tests.

METHODS.—The pea aphid was selected for these tests because it is one of the most important agricultural pests, it is readily available during a large part of the year, and it can be easily reared in the laboratory. Its small size may appear at first to be a disadvantage but the olfactometer used in the laboratory tests could accommodate the species without modification and handling could be

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accomplished without injury by using a camel's-hair brush.

The laboratory-reared aphids were started from 12 adult females collected from alfalfa. They were reared in a constant temperature room at 20° C. on English peas, *Pisum sativum*. The peas were planted in 10- by 12-inch aluminum flats in a mixture of one-half vermiculite and sand. Light for plant growth was supplied by four 40-watt, 48-inch fluorescent bulbs, placed 6 inches above the plants. When the English peas had reached a height of 4 inches the pea aphids were added by means of a camel's-hair brush.

Crowding of pea aphids results in an abundance of winged forms (Comstock 1949) so when winged forms were needed in these experiments the pea plants were allowed to become overpopulated with aphids.

The olfactometer used in the laboratory experiments has been previously described (Chamberlain 1956). Since this experiment was designed to critically evaluate possible field and laboratory conditions only four chemicals were tested as repellents. All four had a previous history of repellent action. The first chemical, methyl pentanone, is commonly used in pea aphid surveys (Cook 1955). Methyl pentanone causes the aphids to loosen their hold on the plants and fall on a board where they may be counted. The second material, camphene, has a high index of repellency to *Melanoplus femur rubrum* in laboratory tests (Chamberlain 1956). The third chemical, 10-undecenoic acid, is strongly repellent to insects of man (King 1954). The last material, paradichlorobenzene, has been used for many years as a moth repellent.

Each of the above chemicals was tested in the olfactometer at three concentrations. The concentrations used for paradichlorobenzene and methyl pentanone were 0.8, 1.6 and 2.4 milligrams per liter of air while 10-undecenoic acid and camphene were tested at 1.6, 2.4 and 3.2 milligrams per liter of air. These concentrations could be maintained by variation in the dilution of the odorized air streams with unodorized air. Some slight variation in the concentration from one experiment to another was expected but little was actually experienced. Each complete test involved four replicated runs with the olfactometer. After the initial 5-minute equilibration period, readings were made every 2 minutes for a period of 10 minutes. After the sixth reading the odorized stream of air was changed to the opposite port and six more 2-minute readings were made after a 5-minute equilibration period. This procedure completed a replicate. Each laboratory replicate involved 20 adult insects either winged or wingless. Most of the tests were conducted using only two groups of 20 insects for the four replicates with an alternation and single re-use of each cage but the effect of only two test groups was one of the variables studied in these tests.

In order to determine how long the test chemical would remain repellent a slight modification was made in the olfactometer equipment. The jar containing the test chemical was replaced by a glass U-tube. The tube was made of 2-cm. glass tubing and had a height of approximately 7 inches and an outside width of 3 inches. In testing the length of repellency of camphene or 10-undecenoic acid two pieces of filter paper measuring 5 by 2 inches were treated with 0.3 ml. of solution. The paper was then inserted into both sides of the U-tube completely covering the top portion. After connecting the U-tube to the olfactometer apparatus and equilibration, a single reading was taken. This procedure was repeated every 30 minutes until no repellency existed. Between tests air continued to flow through the U-tube, but the insects were removed to prevent anesthetization.

Since paradichlorobenzene is a crystalline material, it was dissolved in acetone to make a 3% solution and sufficient material was applied to give 0.3 gm. of paradichlorobenzene per filter paper. When the acetone evaporated from the filter paper, the papers were put into both sides of the U-tube.

Because methyl pentanone was the most volatile of the materials tested the size of the filter papers was increased to 10 by 4 inches and 0.6 ml. of methyl pentanone was added to each filter paper. Before inserting into the U-tube each paper treated with methyl pentanone was folded three times rather than following the system of lining the inside walls of the U-tube as with the other test chemicals. Readings on the methyl pentanone tests were also made continuously every 5 minutes until the repellency ceased to exist.

The repellency index was calculated according to the formula

$$I = \frac{(R - A)}{N} 100,$$

where I is the index, R is the total number of insects on the side of the olfactometer having no odor, A is the total number of insects on the side with the odor and N is the total number of insects times the number of recordings taken.

Field tests were conducted using paradichlorobenzene, camphene and methyl pentanone prepared as 25% dusts. Because severe burning of alfalfa foliage was experienced with a 25% dust of 10-undecenoic acid, this material was prepared as a 10% dust for the field tests. Attaclay was used as the diluent for all the dust preparations. Preparation of the dusts was facilitated by dissolving the test chemical in acetone and, after mixing with the attaclay, allowing the acetone to evaporate. All dust preparations were mixed in a roller mill.

The dusts were applied by means of a L-P Midget Duster to a small field of alfalfa infested with pea aphids. Plots were 20 by 20 feet, replicated four times and randomized. As near as possible, the dusts were applied at the rate of 40 pounds to the acre. Counts of the aphids on each plot were taken before dusting and every 24-hours for 5 days. Five sweeps with a 14-inch insect net were taken on each plot.

RESULTS.—The results of the olfactometer tests conducted to determine the effect of various factors on repellency of chemicals to the pea aphid are shown in table 1. Paradichlorobenzene was more repellent toward the winged forms of *M. pisi* than to the wingless forms. Camphene was slightly more repellent to the wingless form at the 1.6 or 3.2 mg. per liter per minute concentration but at 2.4 mg. no difference existed. No important difference existed between winged and wingless aphids when tested against 10-undecenoic acid. Methyl pentanone was more repellent to the wingless form than to the winged form at all concentrations tested.

Table 1.—Effect of various factors on repellency of the pea aphid in olfactometer tests.

Material	Average Amount of Chemical (mg./l./min.)	Repellency Index: Laboratory-Reared, Winged (Two Cages)[a]	Laboratory-Reared, Wingless (Two Cages)[a]	Laboratory-Reared, Wingless (Four Cages)[b]	Field-Collected Wingless
PDB	0.8	23. ±1.1[c]	13 ±2.7	21 ±2.	21. ±1.1
	1.6	46. ±3.1	31 ±2.2	39 ±1.2	18. ±2.6
	2.4	47. ±4.3	30. ±2.0	44 ±1.9	28. ±1.4
Camphene	1.6	19. ±0.8	38. ±2.9	49 ±1.8	21. ±1.8
	2.4	24. ±1.6	26. ±3.3	36. ±2.2	19. ±1.2
	3.2	21. ±0.9	40. ±1.4	38. ±2.9	35. ±2.7
10-undecenoic acid	1.6	4. ±4.4	13. ±2.4	14. ±3.4	11. ±3.7
	2.4	7. ±5.2	20. ±1.8	13. ±1.2	17. ±2.2
	3.2	26. ±1.4	30. ±1.1	31. ±3.1	22. ±1.9
Methyl pentanone	0.8	21. ±1.	43. ±1.6	19. ±0.8	23. ±1.1
	1.6	38. ±0.9	46. ±0.7	24. ±3.2	34. ±0.8
	2.4	42. ±1.5	53. ±0.6	40. ±1.1	50. ±1.6

[a] Each cage used twice.
[b] Each cage used only once.
[c] Standard error.

The effect of laboratory rearing or field collecting is also shown in table 1. Wingless forms of the field-collected aphids were used in two test cages. Little difference existed between the responses of laboratory-reared and of field insects to the repellents with the possible exception of a greater repellency exhibited by the laboratory-reared insects to methyl pentanone at the two lower concentrations. These data are important because they show that a difference in vitality could not account for differences in results obtained between laboratory and field tests.

In order to determine whether re-using the same insects after a "rest" period had any effect on the results obtained with the olfactometer an experiment was conducted using four different cages of pea aphids each used once. A somewhat variable effect is shown by the results given in table 1. With paradichlorobenzene the four test cages gave a higher repellency index than the test where each cage was used twice, while with methyl pentanone the test where each cage was used twice resulted in a higher repellency index. Tests with camphene and 10-undecenoic acid were inconclusive.

Paradichlorobenzene, camphene and methyl pentanone are rated about equal by the simple type of olfactometer tests. In olfactometer tests 19-undecenoic acid rated considerably below the other three as a repellent for the pea aphid.

Another factor which probably has the most effect on the field value of an agricultural repellent is how long the treated plants will remain repellent. When the four repellents tested were exposed to a continuous flow of air in the olfactometer great variation in the length of repellency resulted as shown in figure 1. All of these tests were conducted with wingless aphids. The camphene-coated strips of filter paper were repellent for a longer period than any of the other test strips, remaining repellent for 4½ hours. Methyl pentanone, rapid evaporation of which was expected, had the shortest period of repellency, only lasting 40 minutes, while paradichlorobenzene and 10-undecenoic acid-coated strips were repellent for 2½ and ½ hours, respectively.

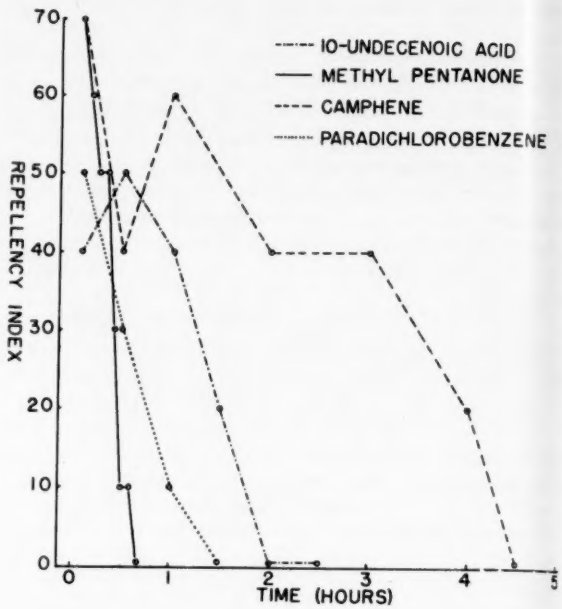

FIG. 1.—The response of the pea aphid, *Macrosiphum pisi* (Harris) to chemical repellents.

The most readily discernible result of the field tests, shown in table 2, is that none of the repellents tested materially reduced the populations of the pea aphid.

DISCUSSION.—The best explanation for the apparent difference between the results of laboratory and field tests is found in the data on the length of repellency of the treated strips of filter paper. None of the strips tested remained repellent for more than 4½ hours. It was 24 hours after treatment of the field plots before the first counts were taken. If the environmental conditions in the field and in the U-tubes in the laboratory were the same, the much longer period of time elapsing in the field tests could explain fully the results obtained. Undoubtedly the environmental conditions were not the same. Records of weather conditions showed that during the 5-day field test the temperature varied from 60° to 83° F., there was no rain and the wind velocity varied from 2 to 20 miles per hour. Calculations show than when 1.6 liters of air per minute flow through the U-tube the velocity is 16.8 feet per minute or 0.19 miles per hour. This means that the flow of air in the olfactometer was not excessive

Table 2.—Field tests with repellents on pea aphids infesting alfalfa.

Material	Average Number of Pea Aphids per Five Sweeps on Days Indicated After Treatment: 1	2	3	4	5
PDB	125±37.3[a]	114± 1.3	95± 2.5	88± 3.5	78± 5.2
Camphene	132± 7.5	110± 3.6	106± 5.2	95± 3.5	79± 1.6
10-undecenoic acid	146± 2.2	62±15.4	57±12	51±11.9	55±14.9
Methyl pentanone	136± 8.3	97±10.8	93± 8.1	83± 5.7	72±13.4
Untreated check	146±12.1	116± 4.5	107± 5.0	79± 3.1	78± 7.6

[a] Standard error.

by comparison with the wind velocities recorded in the field. It would be expected that the repellents were lost from the foliage in less than 4½ hours based on wind velocities recorded. Since the temperature in the laboratory averaged slightly higher than the average daytime temperature in the field, the repellents would be lost at a slightly higher rate from the filter paper strips. When these factors are considered together it is very possible that the maximum period of field repellency would be very close to 4 or 5 hours for the repellents tested.

It is possible that the initial concentration of the repellent on or near the alfalfa plants was not as high as that needed in the olfactometer tests in order to produce repellency. It is rather difficult to calculate, with any degree of accuracy, the actual concentration of the repellents either on or near the leaf surface of the alfalfa plants. It it is assumed that all of the material applied remained in the areas treated for at least a few minutes, and the depth of the treated area was the depth of the alfalfa, it is possible to calculate the average concentration. Since most of the repellents were applied at the rate of 10 pounds per acre and the alfalfa had a depth of approximately 1 foot, the average concentration of the repellents was 3.6 mg. per liter of air. This concentration gave a measurable repellency in the laboratory test. Since our assumptions may or may not be true the above calculated concentrations only indicate that there may have been an initial repellent action by the materials tested.

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DDT Residues on Peach in Ontario[1]

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ABSTRACT

In an experiment on Elberta peach a DDT residue approximately 30% below the official tolerance of 7 p.p.m. was left on the fruit at harvest by the spray schedule recommended for control of the oriental fruit moth (*Grapholitha molesta* (Busck), Olethreutidae: Lepidoptera), namely, 2 pounds of 50% wettable powder per 100 imperial gallons on July 4 and 15 and 1 lb. on August 22, 25 days before harvest. Approximately 4.5 gallons per tree per application were applied. The tolerance was exceeded when three earlier sprays (May 27, June 5 and 17) were added to this schedule. The last spray alone (August 22) left a residue on the fruit at harvest of approximately 25% of the official tolerance permitted.

DDT residue on peaches after harvest has been a potential problem since 1946, when this insecticide was first recommended for the control of the oriental fruit moth, *Grapholitha molesta* (Busck), in Ontario. Dustan *et al.* (1951) showed that from 1947 to 1949 the DDT residue on the fruit at harvest sometimes exceeded 7 p.p.m. following the recommended spray schedule; this consisted of two sprays of 50% wettable powder per 100 imperial gallons 10 to 14 days apart in late June or early July and one spray at 1½ pounds from 3 to 4 weeks before harvest. Brunson & Koblitsky (1950) in New Jersey used 50% DDT at 2 pounds per 100 U. S. gallons in each of three sprays timed similarly to those recommended in Ontario and found residues on peaches at harvest above 7 p.p.m. in two out of nine experiments. Dustan & Boyce (unpublished) found in 1950 and 1951 that when 1 pound of 50% DDT per 100 gallons was applied in the last spray at 3 to 4 weeks before harvest, the residue at harvest was below 7 p.p.m.; however, as Dustan *et al.* (1950) reported, this rate did not always give good control of the oriental fruit moth on nearly ripe fruit.

Fahey *et al.* (1957) reported harvest residues on peach in Indiana of 24.6, 17.4, 11.9, and 5.3 p.p.m. of DDT when the last application of 50% wettable powder at 2 pounds per 100 U. S. gallons was 10, 20, 30, and 40 days, respectively, before harvest. However, the number of applications of DDT in their experiments varied from six to nine per plot, starting at the petal-fall stage of development. They did not state the amounts of materials applied to the trees.

When spray recommendations for peaches in Ontario were revised in 1957 after the establishment of the official tolerance for DDT of 7 p.p.m., it was decided from the available data in Ontario that the interval between the last spray and harvest need not be 42 days as recommended in parts of the United States, but should be 25 days, provided that only three sprays were applied and the last did not exceed 1 pound of 50% DDT per 100 gallons of water.

This is a report on the results of an experiment in the Niagara Peninsula in 1957 conducted to assess the residue factor of various numbers of DDT sprays, the last of which was applied 25 days before harvest.

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METHODS.—The experiment was conducted in a mature Elberta peach orchard. The sprays were applied to unreplicated, 24-tree plots by means of a high-pressure hydraulic sprayer with a hand-operated six-nozzle broom. Spray dates and amounts of DDT applied were as follows:

Dates	*Gallons per Tree*	*50% DDT per Acre (Lb.)*
May 27 (first curculio)	2.8	6.1
June 5 (second curculio)	3.8	8.3
June 17 (special)	3.8	8.3
July 4 (first cover)	4.3	9.4
July 15 (second cover)	4.8	10.5
Aug. 22 (third cover)	4.4	4.8

Fifty per cent captan wettable powder, 2 pounds per 100 gallons, was applied to all plots on August 22 for disease control, and 15% parathion wettable powder for the control of the oriental fruit moth was applied at 1½ pounds on July 4, 15 and August 22 to the plots not receiving DDT on those dates.

Samples of fruit were collected for analysis on September 1, and at harvest on September 16. Four 6-quart samples were collected from 11 to 14 centrally located trees in each plot, the fruit for each sample being taken proportionally from all the trees and half being taken from the upper and half from the lower parts of the trees. The samples were shipped in regular 6-quart fruit baskets to the Kentville laboratory, where analysis was carried out 4 to 8 days after the samples were collected. The four samples from each plot were analyzed separately by the modification of the Schechter method as given by Downing & Norton (1951).

RESULTS AND DISCUSSION.—The maximum of six DDT sprays left a residue after harvest that exceeded the tolerance of 7 p.p.m. by approximately 60% (table 1). This schedule is sometimes followed by a very few growers who add DDT for oriental fruit moth control to the curculio sprays (May 27 and June 5) and make a special application (June 17) against the tarnished plant bug (*Lygus lineolaris* (P.deB.)). When the last spray (third cover) was omitted from this schedule, the residue was below 7 p.p.m. at harvest.

The residue left by the standard three-spray schedule was approximately 60% above the tolerance on September 1, one week after the last application, but had dropped to approximately 30% below the tolerance by harvest on September 16. The residue from the third cover spray alone was approximately one-half the permitted tolerance on September 1, and one-quarter at harvest, 25 days after the spray was applied.

The rate of weathering of the residue was presumably lower in 1957 than usual, for precipitation was generally below the 30-year normal for the district. Inches of rainfall and (in parentheses) these values expressed as percentages of the normals for the corresponding periods were: May 27–June 5, 0.45 (52); June 6–17, 0.24 (21); June 18–July 4, 4.15 (282); July 5–15, 0.57 (68); July 16–August 22, 2.25 (78); and August 23–September 16, 1.63 (62).

The amounts of DDT applied per acre in this experiment were approximately the recommended ones but many growers apply less. It was concluded that it would not be necessary to increase the interval of 25 days between the last spray and harvest of peaches in order to comply with the residue tolerance for DDT provided that the recommended three-spray schedule was followed. Confirmatory evidence for this conclusion was provided by the Food and Drug Directorate of the Canada Department of National Health and Welfare in a letter dated January 24, 1958, stating that of 277 samples of peaches taken after harvest from the 1956 and 1957 crops only three exceeded the residue tolerance of 7 p.p.m. of DDT, and these three had been sprayed with DDT only 4 days before harvest, contrary to the recommendation.

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Table 1.—DDT residues on Elberta peach on two dates after being sprayed as shown.[a]

Dates of Sprays and Numbers of Days Before Harvest						DDT Residue (p.p.m.)			
						Sept. 1		At Harvest Sept. 16	
May 27 112	June 5 103	June 17 91	July 4 74	July 15 63	Aug. 22 25	Range	Mean	Range	Mean
x	x	x	x	x	x	9.0–11.9	11.2±1.40[b]	8.1–11.3	9.7±1.37[b]
x	x	x	x	x		5.4– 7.7	6.6±0.95	5.1– 5.6	5.3±0.22
			x	x	x	9.5–12.2	11.2±1.20	3.5– 5.7	5.0±1.00
					x	2.7– 3.8	3.4±0.50	0.6– 2.3	1.6±0.77

[a] 2 lb. of 50% DDT wettable powder per 100 imp. gal. to July 15; 1 lb. on August 22.
[b] Standard deviation.

Mechanism of Residue Loss of Hercules AC-528 Components on Plant Foliage[1]

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Abstract

The acaricide and insecticide, Hercules AC-528 (Delnav), is a mixture of the *cis* and *trans* isomers of 2,3-*p*-dioxanedithiol *S,S*-bis(*O,O*-diethyl phosphorodithioate) along with certain other phosphorodithioates. Loss of the Hercules AC-528 components from plants resulted from volatilization, hydrolysis, and the formation of more polar derivatives and more potent anticholinesterase agents. The *cis* and *trans* isomers of Hercules AC-528 were similar in persistence so that the isomer ratio did not change during residue loss from that of the insecticide initially applied to the plants. No inter-conversion occurred on plants between the *cis* and *trans* isomers and the dioxene derivative, which is an impurity in the technical insecticide. The components of Hercules AC-528 were only slowly hydrolyzed on the plant surface, but rapidly hydrolyzed when absorbed into the plants. Several of the components were converted to more polar derivatives and more potent anticholinesterase agents when applied to plants. This conversion may be due in part to the formation of phosphorothiolate derivatives. Attempts at chemical oxidation of certain components of Hercules AC-528 to yield the phosphorothiolate derivatives were only partially successful. Several components of technical Hercules AC-528 and certain non-hydrolyzed derivatives formed from the Hercules AC-528 components after application to plants are not determined by the residue analysis procedure of Dunn (1958). The toxicological significance of the organophosphates present in residues of Hercules AC-528 on crops but not determined by the colorimetric method of analysis cannot be fully evaluated with the limited data presented here.

Hercules AC-528 (Delnav) is an acaricide and insecticide of long residual effectiveness (Diveley & Lohr 1955, Diveley *et al.* 1958). The technical product is a mixture of the *cis* and *trans* isomers of 2,3-*p*-dioxanedithiol *S,S*-bis(*O,O*-diethyl phosphorodithioate) along with certain other phosphorodithioates (Diveley *et al.* 1958, Casida & Arthur 1958). The *cis* is more toxic than the *trans* isomer of Hercules AC-528 to mites (Diveley *et al.* 1958) and to house flies and rats (Casida & Arthur 1958)

Analysis of crop residues of Hercules AC-528 has been satisfactorily accomplished by a specific colorimetric method (Dunn 1958). The residues are extracted from the crop with hexane or hexane-isopropyl alcohol, cleaned-up by combinations of adsorption on alumina and partitioning between such solvents as acetonitrile and hexane, cleaved quantitatively to glyoxal and then converted to the 2,4-dinitrophenylosazone which in basic solution yields an intense blue color. Using this procedure, Gunther *et al.* (1958) have shown that the half-life of persisting Hercules AC-528 residues on field-sprayed navel oranges and lemons is about 95 and 85 days respectively. They also demonstrated that emulsifiable formulations of Hercules AC-528 displayed only a persistence type of residue, whereas wettable-powder formulations showed both degradation and persistence residue behavior.

Radiotracer, chromatographic and anticholinesterase techniques were used in this study to determine the persistence and mechanism of degradation of Hercules AC-528 components applied to plants.

Experimental.—*Hercules AC-528 Components Used and Chromatographic Procedures.*—Hercules AC-528-P^{32} was prepared and purified as previously reported (Casida & Arthur 1958). The four radioactive fractions used in this study were the *cis* and *trans* isomers of 2,3-*p*-dioxanedithiol *S,S*-bis (*O,O*-diethyl physphorodithioate), the dioxene derivative (2-*p*-dioxenethiol *S*-(*O,O*-diethyl phosphorodithioate)), and fraction I-P^{32} which was probably bis(diethoxyphosphinothioyl) disulfide (Casida & Arthur 1958). These radioactive fractions are referred to in future discussion as the *cis* and *trans* isomers, dioxene derivative, and fraction I-P^{32}, respectively.

The non-radioactive components of Hercules AC-528 varied from the radioactive only in that fraction I was a mixture of three components (Ia+Ib+Ic) and the components of Hercules AC-528 eluting from a celite-*n*-heptane-acetonitrile column with acetonitrile (fraction V) and with methanol (fraction VI) were used as a mixture (V plus VI) (see Casida & Arthur 1958).

The adsorption and partition chromatographic techniques used, and methods involved in infrared analysis and determination of the extent of hydrolysis through partitioning between chloroform or *n*-hexane and water have been previously described (Diveley *et al.* 1958, Casida & Arthur 1958).

Residue Loss by Volatilization.—The rate of volatilization was determined for radioactive Hercules AC-528 and its components from glass and leaf surfaces. Acetone solutions containing 10 μgm. of each organophosphate were pipetted into 1-inch-diameter glass planchets. Uniform seedling lima bean plants (Fordhook variety) and cotton plants (Delta Pine 15 variety) were also treated with these Hercules AC-528 components. One leaf per plant was painted on the upper surface with 5 μgm. of the radioactive organophosphate in 0.1 ml. acetone to make a radioactive spot one-half inch in diameter. Radioassays on the leaves attached to the plants were made by centering the radioactive spot at the end of an aluminum cylinder mounted around the Geiger-Müller tube and extending 1 inch beyond the mica window. The aluminum cylinder served both to minimize background radiation and permit a reproducible orientation of the radioactive source in respect to the detector. The treated plants and planchets were replicated three times and held in a green-

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Table 1.—Volatilization rate of certain radioactive Hercules AC-528 components at 28° C.

VOLATILIZATION CONDITIONS	APPARENT K[a] FOR FIRST ORDER LOSS CURVES (DAYS^{-1}).	
From glass surface	K_1	K_2
frac. I-P^{32}	0.075	0.033
dioxene	1.8	.057
trans	.032	.018
cis	.030	.015
From cotton foliage		
frac. I-P^{32}	0.10	0.066
dioxene	.77	.043
trans	.030	—
cis	.036	—
From bean foliage		
frac. I-P^{32}	0.13	0.090
dioxene	.77	.055
trans	.030	—
cis	.026	—

[a] Apparent first order rate constants for the loss by volatilization. Where a change in rate occurred during the experimental period the separate rates are indicated as K_1 and K_2.

house at about 28° C. Radioactive measurements were made after 0, 1, 2, 4, 8 and 12 days with the plants and 0, 1, 2, 4, 6, 8, 12 and 20 days with the planchets. Linear responses were obtained when the logarithm of the per cent radioactivity remaining (after correction for isotope decay) was plotted against time. Apparent first order rate constants (k) for the loss were therefore calculated (table 1).

The *cis* and *trans* isomers of 2, 3-*p*-dioxanedithiol *S,S*-bis(*O,O*-diethyl phosphorodithioate) were almost identical in their rate of loss by volatilization. Half-lives varied from 19 to 27 days for the two isomers from a glass surface and from cotton and bean foliage (table 1). Fraction I-P^{32} was two to four times more volatile, while the dioxene derivative was 60 times more volatile from glass and 20 to 30 times more volatile from the plant surfaces than the *cis* and *trans* isomers. When mixtures of the various radioactive Hercules AC-528 components were applied to glass or plant surfaces, the change in slope of the volatilization curves was consistent with the composition and volatility of the components.

Although only limited data were available from the volatilization studies for fitting to first order loss curves, the data when plotted appeared to indicate a break in the volatilization curve or a change in the rate of volatilization with each Hercules AC-528 component from a glass surface. Two components to the volatilization curve (indicated in table I as K_1 and K_2) also appeared with the fraction-I-P^{32} and dioxene derivatives from plant surfaces. The volatilization rate of the Hercules AC-528 components from a glass surface was compared with that of radioactive Thimet, aldrin and DDT. Thimet was more volatile than any of the Hercules AC-528 components, aldrin was slightly more volatile than the dioxene derivative, and DDT was only one-seventh as volatile as *cis* and *trans* Hercules AC-528.

Residue Loss by Hydrolysis.—The rate of hydrolysis of radioactive Hercules AC-528 and its components was determined in dilute alkali and after application to plant surfaces (table 2). Alkaline hydrolysis rates were determined by incubating each radioactive Hercules AC-528 component at 10 p.p.m. in a mixture of 10% ethanol and 90% 0.1 molar aqueous sodium carbonate. After various times of incubation at 28° C., aliquots were removed and extracted with equal volumes of chloroform. A linear relationship resulted with all components when the logarithm of the per cent radioactivity recovered in the chloroform phase was plotted against time. Fraction I-P^{32} was rapidly hydrolyzed under these conditions, and the dioxene derivative was hydrolyzed at three to four times the rate found with *cis* and *trans* Hercules AC-528. The change in hydrolysis rate for mixtures of the radioactive components was consistent with the stability of the constituents of the mixture.

Seedling bean, cotton and tomato (Wisconsin No. 55 variety) plants were treated with 100 p.p.m. of each of the radioactive Hercules AC-528 components. Treatment was accomplished by spraying the plants with water containing 0.5% each of the radioactive organophosphate and Triton X-155. Representative leaf samples were harvested at 0, 1, 2, 4, 8, 12, 20, 28, and 36 days after treatment. The leaf samples were ground in a mortar and pestle with white sand. Samples were ground first with 5.0 ml. *n*-hexane and then with 5.0 ml. water and the combined *n*-hexane, water, sand and leaf residue transferred to 15 ml. centrifuge tubes. The tubes were shaken to extract the unhydrolyzed radioactive compounds into the *n*-hexane, centrifuged, and the radioactivity in each layer determined. As with the alkaline hydrolysis, a linear relationship resulted with all the components when the logarithm of the per cent recovered in the *n*-hexane phase was plotted against time. The *cis* and *trans* isomers of Hercules AC-528 were the most stable to hydrolysis when applied to plant foliage (table 2). Hydrolysis of the fraction I-P^{32} and dioxene components occurred at over 13 times the rate of either the *cis* or *trans* isomers. On cotton and bean foliage the *cis* and *trans* isomers were hydrolyzed at almost the same rate, while on tomato foliage the *cis* was more rapidly hydrolyzed than the *trans* isomer.

It was shown in several different ways that the hydrolysis of the Hercules AC-528 components after application to plants occurred primarily with the organophosphate which was absorbed by the plants. No hydrolysis occurred with any of the radioactive Hercules AC-528 components on exposure to sunlight for 4 days in a thin film on a glass surface. When the persisting metabolites on the plant surface were removed by thoroughly washing the treated foliage with chloroform, less than 1% hydrolysis products were found in these surface residues. This failure of

Table 2.—Hydrolysis rate of certain radioactive Hercules AC-528 components at 28° C.

HYDROLYSIS CONDITIONS	APPARENT HYDROLYSIS CONSTANT (K) FOR:			
	Frac. I-P^{32}	Dioxene	*Trans*	*cis*
In 0.1 *M*. Na_2CO_3 (min.$^{-1}$)	g.t. 7	0.32	0.11	0.077
On plant foliage (days^{-1})				
cotton	1	0.3	0.014	0.014
bean	0.86	.4	.028	.027
tomato	.53	.32	.013	.024

Hercules AC-528 components to hydrolyze on the surface of the plant foliage was demonstrated with cabbage harvested 0, 2, 8 and 16 days after foliar treatment with radioactive technical Hercules AC-528, with cabbage harvested 0, 4 and 12 days after treatment with either *cis* or *trans* Hercules AC-528, and with beans harvested two day after treatment with the dioxene derivative. The rapid hydrolysis of Hercules AC-528 fractions in plants was further demonstrated with bean seedlings which had absorbed the radioactive components through the roots from water containing 40 p.p.m. each of the radioactive component and Triton X-155. Fraction I-P^{32} was the least stable, followed by the dioxene derivative and the *cis* and *trans* isomers were the most stable after absorption by the beans. After 1.5 days absorption time through the roots, the *cis* and *trans* isomers in the foliage were about 70% hydrolyzed and this increased to 87% after 4 days absoprtion and metabolism time. In absorbing the radioactive dioxene derivative through the roots of beans, the solution level of dioxene derivative dropped from 40 p.p.m. initial to 20 p.p.m. after 4 days with corresponding increase in the plant radioactivity. Little or no hydrolysis occurred in the solution surrounding the roots or in the roots and only the portion of the dioxene derivative translocated into the foliage was hydrolyzed.

Possible Inter-conversion of Hercules AC-528 Components on Plants.—Because of the long persistence of the Hercules AC-528 components on plants, the possibility exists that certain inter-conversions might take place such as the conversion of one geometrical isomer to the other or the degradation of one of these isomers to the dioxene derivative. Thermal degradation of the *trans* isomer forms the dioxene derivative in good yields (Diveley *et al.* 1958). Also when technical Hercules AC-528 (non-radioactive) was exposed to sunlight in thin films on glass surfaces at 28° C. for 0, 2, 8 and 32 days, a slight increase in the ratio of *cis* to *trans* isomer occurred based on the infrared spectra of the recovered Hercules AC-528.

The nature of those Hercules AC-528 derivatives which were soluble in organic solvents was studied with bean and cabbage (Globe variety) seedlings treated at 150 p.p.m. by spraying them with water containing 0.5% each of the radioactive organophosphate and Triton X-155. At several times after treatment plants were harvested and the samples quick-frozen and held at −10° C. until analyzed. Each 100-gm. foliage sample was macerated with 200 ml. chloroform in a Waring Blendor, and the insoluble plant materials removed by straining through cheesecloth. The chloroform and aqueous layers were then separated and the chloroform layer filtered through anhydrous sodium sulfate. The solid plant residue was washed with acetone until no further radioactivity was recovered; the acetone was then evaporated and the residue dissolved in chloroform and partitioned twice with equal volumes of water. This chloroform was dried with anhydrous sodium sulfate and added to the original chloroform extract. The radioactive materials extracted with chloroform from plants treated with the dioxene derivative were then chromatographed on celite with carrier non-radioactive Hercules AC-528 (Casida & Arthur 1958). With the plants treated with the other three radioactive Hercules AC-528 components, further clean-up of the chloroform-soluble materials was effected with alumina columns and the per cent of the total radioactivity recovered in the *n*-hexane, benzene and methanol fractions was determined. The radio-active benzene fractions were combined and chromatographed on a celite column with carrier non-radioactive Hercules AC-528. The zero-day results were obtained by adding the radioactive component to the chloroform and untreated plant tissue in a Waring Blendor and following the same extraction, clean-up and chromatographic procedures.

No conversion occurred on plants of one geometrical isomer to the other nor did either isomer form any dioxene derivative or other less polar metabolites. This conclusion is based on analyses of beans 10 days after treating with *trans* Hercules AC-528, cabbage at 0, 4, and 8 days after treating with *cis* Hercules AC-528, and beans and cotton at 10 and 20 days after treating with *cis* Hercules AC-528. None of the radioactive components from plants treated with the dioxene derivative was eluted from celite columns with *n*-heptane equilibrated with acetonitrile in a position other than that for the known dioxene derivative. This was shown for beans and cabbage at 0, 2, 4 and 8 days after treatment, and for both the surface wash and absorbed material 2 days after treatment of beans. Also, none of the radioactive components from cabbage foliage treated with fraction I-P^{32} at 0 or 2 days after treatment was eluted from celite columns with *n*-heptane equilibrated with acetonitrile in a position other than that for the known fraction I-P^{32} component.

In a larger scale experiment, 7.5 kg. of bean seedlings were treated with technical Hercules AC-528 to yield a 200 p.p.m. initial residue. Twelve days after treatment the plants were harvested, extracted with chloroform, and the chloroform-solubles cleaned-up by hexane-acetonitrile partitioning, chromatography on a celite column with *n*-heptane-acetonitrile, then chromatography on an alumina column and finally with activated charcoal. The final product recovered (71 mg.) was identical in the ratio of the *cis* and *trans* isomers of Hercules AC-528 based on an infrared spectrogram with the original technical Hercules AC-528 applied. Since the *cis* and *trans* Hercules AC-528 isomers were found in preliminary studies to be recovered to the same extent with this clean-up procedure, it appears that no conversion of one geometrical isomer to the other occurred on the bean plants to change the *cis* and *trans* isomer ratio.

Formation of More Polar Derivatives on the Plant Surface.—The effect of sunlight on the solubility properties of the radioactive Hercules AC-528 components was studied by exposing thin films of the components (about 60 μgm. per sq. in.) on glass surfaces in a greenhouse at 28° C. for 0, 2, 4 and 8 days. The radioactive materials were recovered by dissolving in acetone, spotted on Whatman no. 1 chromatographic paper and the paper chromatograph developed by the reversed phase system of Cook (1954a). The radioactive components were located by radioautograms and the total radioactivity in each spot determined. The R_f values for the radioactive Hercules AC-528 components were 0.13 for fraction I-P^{32} 0.53 for *trans* and 0.55 for *cis* Hercules AC-528, and 0.88 for the dioxene derivative. Small amounts of more polar derivatives were formed on exposure to light. These products gave R_f values of 0.95 to 1.0 and were not hydrolysis products. With *cis* and *trans* Hercules AC-528

Table 3.—Nature of persisting residues of certain Hercules AC-528 components on bean foliage.

Component and Conditions	Per Cent Hydrolyzed	Per Cent Eluted with:[a]		Partitioning Properties[b]	
		Heptane	A-N	Heptane	N-N
Technical Hercules AC-528—					
12 days total foliage	22.8	76.3	0.9	89	56
Dioxene—total foliage					
0 days	0.9	99.1	0.02	79	31
2 days	42.3	48.7	9.0	79	25
4 days	62.2	35.8	2.0	79	15
8 days	74.2	25.0	0.8	78	15
Dioxene—2 days[c]					
surface wash	0.8	47.0	3.5	72	5
leaf extract	37.5	9.5	1.7	73	18

[a] Non-hydrolyzed derivatives chromatographed on celite-*n*-heptane-acetonitrile column which was developed first with *n*-heptane equilibrated with acetonitrile (heptane fraction) and then with acetonitrile equilibrated with *n*-heptane (A-N fraction).

[b] Per cent radioactivity in hexane phase with hexane, acetonitrile, water at 2:1:1.

[c] Calculated on the basis of the total radioactivity in the combined surface wash and leaf extract.

these more polar derivatives constituted 1.8 to 3.7% of the total radioactivity. Up to 3.5% more polar derivatives were formed with fraction I-P_{32} and even higher percentages with the dioxene derivative where difficulty was encountered in obtaining reproducible results because of the rapid volatilization of this Hercules AC-528 component.

Cabbage and bean plants treated with the radioactive Hercules AC-528 components at 150 p.p.m. were extracted as described in the previous section. The formation of more polar metabolites was shown for plants treated with the dioxene derivative by chromatographing the chloroform-soluble radioactivity on celite columns from which the dioxene derivative was eluted with *n*-heptane equilibrated with acetonitrile and the more polar metabolites with acetonitrile equilibrated with *n*-heptane. For plants treated with the *cis*, *trans* or fraction I-P^{32}, the chloroform-soluble radioactive compounds were chromatographed on alumina columns; the radioactive compounds adsorbed on the columns with *n*-hexane and benzene, but eluted with methanol, were considered as more polar metabolites. The partitioning properties of all radioactive fractions from the columns were then determined with *n*-hexane, acetonitrile and water in volume ratios of 2:1:1.

More polar derivatives were formed from each of the radioactive Hercules AC-528 components after application to plants (tables 3 and 4). The metabolites of the dioxene derivative partitioned more completely into acetonitrile-water from *n*-hexane than the metabolites of the *cis* and *trans* isomers. The more polar, non-hydrolyzed products from the dioxene derivative in the surface wash of bean foliage 2 days after treatment, partitioned similarly and had a similar anticholinesterase activity to a known sample of 2-*p*-dioxenethiol *S*-(*O*,*O*-diethyl phosphorothioate). These more polar derivatives formed more readily and disappeared more rapidly with the dioxene derivative than with the *cis* and *trans* isomers of Hercules AC-528. With plants treated with *cis* or *trans* Hercules AC-528 the radioactive materials eluted from the alumina column with methanol partitioned only slightly less into hexane with the hexane-acetonitrile-water system than the materials eluted with benzene (table 4). It is possible that the percentages of more polar derivatives as determined with the alumina column were affected by non-radioactive plant extractives which changed the ease of elution from the columns, but this does not appear to be the entire explanation for the appearance of products which were not eluted with benzene since there was an increase in the proportion of this fraction with time after treatment even though the same plant extractives were present in all cases.

Attempts at Preparation of Phosphorothiolate Derivatives of Certain Hercules AC-528 Components.—Attempts at oxidation of certain Hercules AC-528 components to yield compounds comparable to the more polar derivatives formed when these components are applied to plants were only partially successful. Syntheses were attempted for 2-*p*-dioxenethiol *S*-(*O*,*O*-diethyl phosphorothioate); 2,3-*p*-dioxanedithiol *S*,*S*,bis(*O*,*O*-diethyl phosphorothioate); and 2,3-*p*-dioxanedithiol *S*-(*O*,*O*-diethyl phosphorodithioate) *S*-(*O*,*O*-diethyl phosphorothioate). These compounds are designated for discussion as dioxene-P=O; dioxane-P=O, P=O; and dioxane-P=S, P=O; respectively. The chemical nature of the products formed was evaluated by chromatographic characteristics on celite and alumina, infrared spectra, and the per cent total phosphorus partitioning into *n*-hexane with *n*-hexane, acetonitrile and water in 2:1:1 volume ratios.

Oxidation of the dioxene derivative with equimolar peracetic acid in anhydrous chloroform readily formed the dioxene-P=O in high yields. An infrared spectrum of the dioxene-P=O differed from one for the dioxene-P=S derivative only in an absorption band shift from 9.45 to 9.25 microns, the loss of a band at 15.45 microns, and the formation of a strong P=O absorption band at 8.1 to 8.3 microns. The infrared spectrum of an authentic dioxene-P=O sample (product provided by Dr. W. R. Diveley, Hercules Powder Co.) was identical after purification on

Table 4.—Nature of persisting residues of certain Hercules AC-528 components on cabbage foliage.

Component	Days After Treatment	Per Cent Hydrolyzed	Per Cent Eluted with:[a]		Partitioning Properties[b]	
			Benzene	Methanol	Benzene	Methanol
Frac. I-P^{32}	0	1.7	97.6	0.7	97	61
	2	72.4	11.4	16.2	99	46
Dioxene[c]	0	1.6	98.4	0.02	79	35
	2	60.4	35.4	4.2	80	20
	4	78.6	20.4	1.0	78	24
	8	82.9	16.6	0.5	78	17
trans	0	1.8	97.6	0.6	94	89
	2	7.7	86.7	5.6	92	87
	4	18.5	74.3	7.2	90	83
	8	29.1	65.2	5.7	91	72
	16	46.8	47.6	5.6	88	81
cis	0	0.6	98.9	0.5	93	87
	2	6.9	87.7	5.4	93	87
	4	16.0	77.6	6.4	94	86
	8	24.4	69.5	6.1	95	70
	16	53.7	41.9	4.4	95	80

[a] Non-hydrolyzed derivatives chromatographed on alumina column which was developed with hexane, benzene and methanol in that order. None of the radioactivity in any case was eluted with hexane.

[b] Per cent radioactivity in hexane phase with hexane, acetonitrile, water at 2:1:1.

[c] Celite-*n*-heptane-acetonitrile column developed first with *n*-heptane equilibrated with acetonitrile (this fraction indicated under "benzene" column to which it was comparable) and then with acetonitrile equilibrated with *n*-heptane (this fraction indicated under "methanol" column to which it was comparable).

an alumina column to the product formed by peracetic acid oxidation. The dioxene-P=O derivative was adsorbed on the alumina column with *n*-hexane, benzene and chloroform, but was eluted with acetonitrile or methanol. On the celite column, this dioxene-P=O derivative was not eluted with *n*-heptane equilibrated with acetonitrile, but was readily eluted with acetonitrile equilibrated with *n*-heptane. The dioxene-P=S partitioned 78% into *n*-hexane and the dioxene-P=O only 4% with the *n*-hexane-acetonitrile-water system.

Attempted synthesis of the dioxane-P=O, P=O derivative (Diveley *et al.* 1958) and the dioxane-P=S, P=O derivative (impure product provided by Dr. W. R. Diveley, Hercules Powder Co.) yielded dioxene-P=O as the only phosphorothiolate compound present containing the dioxene or dioxane ring based on infrared spectra of the products after fractionation on alumina and celite columns. Reaction of 2,3-*p*-dichloro-*p*-dioxane with one molar equivalent each of potassium *O,O*-diethyl phosphorodithioate and potassium *O,O*, diethyl phosphorothioate or with two molar equivalents of the latter salt in acetone for 12 hours at 28° C. failed to yield the dioxane-P=S, P=O or dioxane-P=O, P=O derivatives based on infrared absorption spectra of the products.

Reaction of Hercules AC-528 with peracetic acid appeared to be more successful in forming the dioxane-P=S, P=O and dioxane-P=O, −P=O derivatives. Technical Hercules AC-528 was purified on an alumina column (Dunn 1958) and the benzene fraction reacted with one or three molar equivalents of peracetic acid in anhydrous chloroform at 61° C. for 10 minutes. The products in chloroform were washed with aqueous sodium carbonate containing phenolphthalein until the pink color persisted in the aqueous layer. The chloroform-soluble organophosphates were then fractionated on an alumina column using *n*-hexane, benzene, benzene:chloroform at 1:1, chloroform, acetonitrile and methanol elutriants. No phosphorus-containing materials which showed a distinct P=O infrared absorption band were eluted with any solvents except chloroform and acetonitrile. A very small amount of the total phosphorus was eluted from the columns with chloroform, and this fraction had a small but distinct infrared band at 8.1 to 8.3 microns in the position of the P=O absorption. A higher proportion of oxidation products were eluted with acetonitrile, this fraction constituting 15% of the non-hydrolyzed organophosphates with 1.0 molar equivalent of peracetic acid and 2.8% with 3.0 molar equivalents of peracetic acid. The infrared spectra on the products eluted by acetonitrile were identical with the one or three molar ratio of oxidizing agent and showed an absorption peak at 8.1 to 8.3 microns which was as strong as the P=O band appearing in the dioxene-P=O derivative. Technical, *cis* and *trans* Hercules AC-528 all partitioned from 88 to 94% into *n*-hexane with the *n*-hexane-acetonitrile-water system, while the oxidation product eluted with chloroform partitioned 62% into hexane and that eluted with acetonitrile partitioned 37% into hexane. When injected subcutaneously into rats at 20 to 30 mg./kg. the oxidation products eluted with chloroform and acetonitrile produced symptoms of organophosphate poisoning but no mortality. Since these oxidation products displayed the infrared absorption bands between 8.8 and 9.2 microns and between 11.2 and 11.6 microns characteristic of mixtures of the *cis* and *trans* isomers of Hercules AC-528 (Casida & Arthur 1958), it is possible that the material eluting from alumina with chloroform was the dioxane-P=S, P=O derivative, and that with acetonitrile was the dioxane-P=O, P=O derivative as mixtures of the *cis* and *trans* isomers.

Formation of Anticholinesterase Agents After Application to Plants.—Cotton seedlings, 8 inches tall, were treated with technical Hercules AC-528 and each of its components. Treatment was accomplished by dipping the plant foliage into a water emulsion containing 2860 p.p.m. organophosphate and 800 p.p.m. Triton X-100, and using a cotton swab to scrub the leaf surfaces to make sure that the total foliage was wetted with the emulsion. Based on the amount of emulsion used, this constituted treatment with about 1000 p.p.m. of each component when calculated on the initial fresh weight of the foliage. At several times after treatment (table 5) the plant foliage was analyzed for total anticholinesterase agents and in certain cases for Hercules AC-528 by a colorimetric procedure. Aqueous homogenates of the plant foliage were assayed directly for anticholinesterase agents by the method of Casida *et al.* (1956). For colorimetric analysis, 2- to 5-gram leaf samples were soaked with occasional shaking for not less than 24 hours with 60 ml. of 2:1 *n*-hexane-isopropyl alcohol. After washing with water to remove the alcohol, an aliquot of the *n*-hexane solution was analyzed by Dr. C. L. Dunn (Hercules Powder Co.) using his reported method (1958).

A loss of about 80 to 90% occurred with technical, *trans* and *cis* Hercules AC-528 over a 21-day period based on colorimetric analysis. This loss was due in large part to dilution of the insecticide from the rapid plant growth during the 3-week period. The dioxene derivative formed more potent anticholinesterase agents very rapidly (with-

Table 5.—Anticholinesterase activity of cotton plants after treatment with Hercules AC-528 components at about 1000 p.p.m.

Days After Treatment	Technical Hercules AC-528	Fractions from Technical Hercules AC-528[a]				
		I a+b+c	II dioxene	III *trans*	IV *cis*	V+VI
Anti ChE activity[b]						
0 (calc.)[c]	10	6	112	100	>320	5
0.2	19	11	3	48	> 80	8
1	10	11	4	24	9	6
3	7	12	11	11	8	10
7	3	8	12	6	4	12
14	9	11	20	8	6	36
21	16	6	23	8	6	34
P.p.m. Hercules AC-528[d]						
0.2	941	—	—	1100	1470	—
21	174	—	—	203	150	—

[a] Fractions from celite-*n*-heptane-acetonitrile column as previously described (Casida & Arthur 1958).

[b] The μg of plant tissue necessary to 50% inhibit the cholinesterase activity in 1.0 ml. 30% whole human blood with 1.0 hour incubation of plant tissue and blood together at 38° C. before addition of substrate (method of Casida *et al.* 1956).

[c] Calculated from the amount of emulsion used to treat the plants and the anticholinesterase activity of these purified components (Casida & Arthur 1958) under the same assay conditions. These figures represent only an approximation (± 50%) because of the small sample sizes and difficulty in accurately reproducing the method of application.

[d] Determined colorimetrically (Dunn 1958) by Dr. C. L. Dunn, Hercules Powder Company. The technical Hercules AC-528 analysis was calculated on the basis of 70% conversion to glyoxal 2,4-dinitrophenylosazone while that of the *cis* and *trans* isomers was calculated on 100% conversion.

in 0.2 days), but they were also rapidly dissipated. The anticholinesterase agents from fraction $I(a+b+c)$ were very stable on cotton, while those from fraction V plus VI diminished within a few days. With technical, *trans* and *cis* Hercules AC-528, the anticholinesterase activity reached a maximum about a week after treatment and then diminished slowly. Considering these results (table 5) in relation to the proportionate composition of technical Hercules AC-528 (Casida & Arthur 1958), it would appear that the anticholinesterase agents formed from the *cis* and *trans* isomers would be the most persistent of the anticholinesterase agents in or formed from the components of technical Hercules AC-528.

DISCUSSION.—Hercules AC-528 is composed of at least eight different phosphorothioates with the *cis* and *trans* isomers of 2,3-*p*-dioxanedithiol *S,S*-bis(*O,O*-diethyl phosphorodithioate) comprising about 70% of the technical material (Diveley *et al.* 1958, Casida & Arthur 1958). Residue loss of the components is effected by volatilization, hydrolysis, and formation of more polar and/or more potent anticholinesterase derivatives. When only volatilization and hydrolysis were considered, the half-life of *cis*-Hercules AC-528 was 13 days on bean and 14 days on cotton foliage, of *trans*-Hercules AC-528 was 13 days on bean and 15 days on cotton foliage, and of the dioxene derivative was 0.5 days on bean and 0.8 days on cotton foliage. The low vapor pressure and stability to hydrolysis of the *cis* and *trans* isomers of Hercules AC-528 on plant foliage account for their long residual persistence. The persistent insect and mite control from Hercules AC-528 is probably due primarily to the *cis* isomer because of its higher biological activity. No conversion of one geometrical isomer to the other occurred with the *cis* and *trans* isomers on plants nor did they form any dioxene derivative. The *cis* to *trans* isomer ratio held nearly constant after application of Hercules AC-528 to plants. The hydrolysis which occurred with the Hercules AC-528 components appeared to be primarily with the absorbed materials, since surface-washes of treated foliage recovered almost entirely non-hydrolyzed Hercules AC-528 components. When absorbed by plants, the radioactive derivatives studied were rapidly hydrolyzed with the hydrolysis of the *cis* and *trans* isomers being somewhat slower than for the dioxene or fraction I-P^{32} derivatives.

On exposure to sunlight or after application to plants, certain of the Hercules AC-528 components form more polar derivatives and more potent anticholinesterase agents. These derivatives may be phosphorothiolate analogs of certain of the Hercules AC-528 components. It is known that when Thimet is absorbed by plants, the *O,O*-diethyl *S*-ethylsulfinylmethyl and *S*-ethylsulfonylmethyl phosphorodithioate metabolites are slowly oxidized *in vivo* to the phosphorothiolate analogs (Bowman & Casida 1957). Also, many phosphorothioate insecticides are converted in the presence of ultraviolet light to more polar derivatives of high *in vitro* anticholinesterase activity (Cook 1954b, 1955, Cook & Pugh 1957, Frawley *et al.* 1958). The formation of more polar derivatives and more active *in vitro* anticholinesterase agents from certain of the Hercules AC-528 components after application to plants is therefore consistent with the information available from studies on other phosphorothioate insecticides.

The analytical method available for Hercules AC-528 (Dunn 1958) determines only the *cis* and *trans* components of the technical material and fails to account for impurities and metabolites which are removed in purification of the plant extracts on the alumina column or do not cleave with mercuric chloride to yield glyoxal. The toxicological significance of Hercules AC-528 components and derivatives formed after application to plants which are not determined by the analysis method of Dunn (1958) cannot be fully evaluated with the limited data presented here.

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A Pepper-Flower Bud Worm, *Gnorimoschema gudmannella* (Wlsm.)[1]

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ABSTRACT

The Gelechiid, *Gnorimoschema gudmannella* (Wlsm.), was found attacking the flower buds of *Capsium annum* L. in the Lower Rio Grande Valley during 1956. Biological studies showed that the average life cycle was 37.6 days at 80±2° F. and 70 ±3% relative humidity. The female adult laid an average of 154.6 eggs over an average of 11.5 days. Two Braconid parasites, *Origilus* n. sp. and *Chelonus phthorimaeae* Gahan., were found to attack the species.

The pepper-flower bud worm, *Gnorimoschema gudmannella* (Wlsm.), has become one of the major pests of cultivated peppers in the Lower Rio Grande Valley. First damaging infestations of this insect were observed during the fall of 1956. Wilson (1923) reported this insect as a pest of peppers in the Virgin Islands. It was found in Puerto Rico by Sein (1942) who noted it "abundant on buds and flowers of cultivated pepper and aji at Rio Piedras." The insect was first reported in the continental United States from Florida in 1944 and later from Texas in 1945 by Annand (1945). This paper is primarily a study of the life history and seasonal incidence of the pepper-flower bud worm.

DAMAGE AND SEASONAL HISTORY.—In the fall of 1956 bell peppers did not set fruit properly. A close examination revealed larvae of *G. gudmannella* feeding within the flower buds. All varieties of *Capsicum annum* L., including several strains of wild peppers which occur throughout the Lower Valley, were found to serve as hosts of this insect. Furthermore, these wild peppers (called bird peppers locally) served as carry-over hosts through the summer and winter.

The small larva enters the bud and feeds upon the style, anthers and ovary. Usually this causes the bud to drop before maturity. However, if the bud is attacked well toward maturity, it may open but may not be pollinated due to the destruction of the style.

The extent of damage was dependent upon earliness of infestation which in turn was dependent upon the distance of the crop from an infestation in wild peppers. Infestation by this bud worm in a Jalapeno pepper field that was maintained throughout 1957 varied from 70% to 100% infested buds. A normal crop of fruit was set very early during the spring. Thereafter, the infestation of bud worms increased rapidly until July at which time 100% of the buds were infested. Field surveys during the past 2 years indicated that there was a gradual population increase in the fall crop of peppers during September and by October all fields showed some degree of infestation.

LIFE HISTORY.—The life history of the insect was studied on bell peppers growing under screen cages during November 1956 through March 1957. Three generations and a partial fourth were completed during this period. Thirty-two to 51 days were required for a generation.

Life history studies were also conducted in a controlled temperature and humidity room held at 80±2° F. and 70±3% relative humidity. Oviposition cages consisted of glass tubes (48×100 mm.) closed at each end with milk bottle caps. Each tube was set upright with a small branch of pepper stuck through a hole in the bottom cap so that the cut end could be placed into a small dish of water. The pepper branches were changed daily when the eggs were collected and counted. The eggs were placed on potted pepper plants growing in the room. After hatching, the larvae were allowed to enter the buds and begin feeding. Five to six days later the infested bud was removed from the plant and placed into a small stender dish. Fresh pepper buds were added daily until the larvae pupated. Newly emerged adults were fed a mixture of honey and water before being placed into oviposition cages. Adults mated shortly after emergence, and this one mating was sufficient for the fertilization of all eggs although multiple mating was noted. A summary of the life history studies is given in tables 1 and 2.

The freshly deposited egg is opaque and pearly white but as it becomes older changes to a yellowish color. The young larva can be seen through the shell shortly before hatching. Eggs are irregular in shape and are generally deposited along the veins on the leaves and in axils of leaves or branches. The eggs were laid singly or in small groups. The incubation period varied from 3 to 4 days in the laboratory and from 4 to 7 days in the field.

The newly emerged larvae averaged 0.38 mm in length and were extremely active immediately after hatching. The larvae did not attempt to enter buds for the first 4 or 5 hours but spent this time crawling about on the plant. Most larvae did not enter buds until 8 to 10 hours after emergence, but all had entered by 12 hours. Normally, each larva was able to develop to maturity on the food obtained from one bud, although two or more buds were required when buds were less than one-third developed. The mature larvae averaged 3.6 mm. in length. A period of 7 to 13 days was required for development in the laboratory and 14 to 24 days in the field. The prepupae changed to a brick-red color 2 days before spinning cocoons. Cocoons were formed upon the ground by incorporating fine grains of sand into the webbing.

The duration of pupal period in the field studies ranged from a minimum of 11 days to a maximum of 21 days. Under laboratory conditions the length of this period varied from 7 to 10 days. The pupae changed color from light yellow to dark brown just before adult emergence.

Under laboratory conditions female moths lived from 7 to 35 days while male adults lived for a period of 13 days to 35 days. The pre-oviposition, oviposition, and post-oviposition periods ranged from 9 to 19 days, 4 to 22 days and 1 to 11 days, respectively. Female adults laid an average of 154.6 eggs with a minimum of 58 and a maximum of 292 over an average of 11.5 days. Females developed from Bell

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Table 1.—Number of eggs laid by *G. gudmannella* females under controlled humidity and temperature.[a]

	Days of Oviposition												
	1	2	3	4	5	6	7	8	9	10	11	12	
Minimum	2	5	2	0	1	3	0	4	0	0	2	0	58
Average	16.8	19.3	17.6	19.5	12.4	15.3	10.7	12.3	7.5	6.2	6.2	2.7	154.6
Maximum	32	27	41	34	25	45	28	31	21	21	19	8	292

[a] 80±2° F. and 70±3% relative humidity.

pepper buds deposited an average of 68.4 more eggs than those from Jalapeno buds.

During the laboratory study, eight virgin female moths were observed in order to ascertain whether they would reproduce parthenogenetically. No eggs were deposited after a period of 30 days. Four of the moths were then mated and after a short pre-oviposition began to deposit eggs. The remaining unmated females died without depositing eggs.

Table 2.—Number of days required for completion of various stages under controlled humidity and temperature.[a]

	Eggs	Larvae	Pre-Pupa	Pupa	Pre-Oviposition	Total
Minimum	3	7	2	7	9	30
Average	3.5	9.7	2	8.4	14.0	37.6
Maximum	4	13	2	10	19	45

[a] 80±2° F. and 70±3% relative humidity.

INSECT PARASITES.—The Braconid parasites, *Origilus* n. sp. and *Chelonus phthorimaeae* Gahan., were reared from *G. gudmannella*. The parasites emerged from the pupae at about the expected emergence date of the moth. *Origilus* parasitized as high as 30% of the larvae during October and November 1956 while *C. phthorimaeae* was a minor parasite that was only found occasionally.

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Breakdown of Lindane and Aldrin in Soils[1]

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ABSTRACT

A muck soil, Miami silt loam and a sandy loam were treated in 1954 with lindane at rates of 10 and 100 pounds per 6-inch acre and aldrin at rates of 2, 20, and 200 pounds per 6-inch acre. Laboratory experiments were conducted, where a Miami silt loam was treated with either lindane or aldrin at a rate of 10 p.p.m.

Lindane was found to break down within 2 weeks after application to a nontoxic compound, which was still detected by the colorimetric method for lindane, but not by bioassay. Two weeks after treatment the quantity estimated by bioassay amounted to 66% of that obtained by chemical analysis. Even after 3 years the same ratio was found between bioassay and chemical results. When field samples were analyzed, 3½ years after treatment, the quantity estimated by bioassay amounted to 46% of that obtained by chemical analysis.

Aldrin was found to be converted into dieldrin, which first was indicated by higher recoveries obtained by bioassay. Chemical analysis for dieldrin proved the presence of this insecticide in soils. It was found that relatively more dieldrin was formed in a sandy loam and in soils which had been treated at lower concentrations and which were held at higher temperatures.

Experiments conducted in the laboratory showed that 2 weeks after treatment no dieldrin was formed in a loam having been held at 7° C. In soils, having been held at 26° C. or 46° C., 4 to 8% of the recovered insecticides were found to be dieldrin. The peak of dieldrin formation was reached 56 days after treatment, followed by a decrease.

Insecticidal residues are chemicals, which have been exposed for a certain time to various factors. The residues must be derived from the compound, with which the substrate had originally been treated. They may consist of

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either the insecticide itself, breakdown products of the insecticide or both. For the control of insects as well as for agricultural usage of insecticide-treated soils it is, of course, desirable to know how long an insecticide persists. Moreover, it is extremely important to find out if an insecticide breaks down and whether or not its breakdown products are toxic to insects or plants.

When analyses for insecticidal residues are conducted, the analytical method employed is of primary importance. In the case of the chlorinated hydrocarbons the "Total Chlorine" method will not give an accurate picture of the specific insecticide present. Only the total amount of organic cholorine will be measured. On the other hand, a chemically specific method will detect the amount of insecticide present, but in most cases will not give any information on breakdown products. When bioassay procedures are employed, no information concerning the nature of the insecticide is obtained but residual toxicity is measured. Therefore, any toxic breakdown product will be detected by this method. In fact, four possibilities are apparent when both a specific analytical method and a bioassay procedure are employed. If an insecticide *A* partially breaks down to form *B*, the end result is *A*+*B*. If the compound *B* is nonreactive in the chemical method and is nontoxic, the analyses by either method should be essentially identical. However, if compound *B* is still toxic to insects, the bioassay results will be higher than the results obtained by chemical analysis. If compound *B* is nontoxic, but still reactive in the course of a colorimetric analysis, the recoveries obtained by chemical analysis will be higher as compared with the results obtained by bioassay. If compound *B* is both reactive and toxic, the results of the two methods will also be in essential agreement, with whatever disparity, dependent on the relative toxicity of *A* and *B*.

The conversion of aldrin to dieldrin in animal tissues was shown by Bann *et al.* (1956) and has also been demonstrated to occur in soils (Edwards *et al.* 1957, Gannon & Bigger 1958, Bollen *et al.* 1958). Lindane in soils breaks down to a nontoxic product, which will still respond to the Schechter-Hornstein method of analysis (Lichtenstein 1958). Sternburg & Kearns (1956) demonstrated that pentachlorocyclohexene is "an intermediate in the metabolic fate of lindane in house flies."

To obtain more information on the fate of lindane and aldrin in soils, field and laboratory tests were conducted and residue data were obtained by both chemical and bioassay methods.

PROCEDURES.—*Field Experiments.*—In May 1954, a muck soil, Miami silt loam and a fine fox sandy loam were treated in Wisconsin with lindane at rates of 10 and 100 pounds per 6-inch acre and with aldrin at rates of 2, 20 and 200 pounds per 6-inch acre. The insecticidal application involved thorough mixing of an emulsifiable concentrate with 10 gallons of water. Experimental plots (50′×29′) were divided into five 10′×29′ subplots and 2-gallon quantities of the diluted emulsion were spread as uniformly as possible with a sprinkling can over each subplot. Thereafter each plot was rototilled to a depth of 4 to 5 inches. Later on, a rototiller or disc was used to keep the experimental plots in fallow as much as possible.

Samples of the plots treated in 1954 with lindane were collected 3 and 3½ years later, and the aldrin-treated plots were sampled at 2, 3 and 4 years after treatment. A soil auger (¾ inch in diameter by 6 inches long) was used and 40 cores were collected from each plot. The area of each plot was reduced to 21′×42′ for sampling, leaving a 4′ strip between the boundaries of the treated plot and the sampling area.

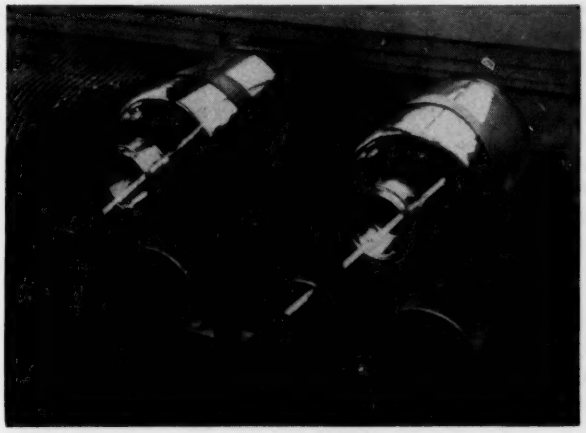

FIG. 1.—Apparatus used for treating and mixing soil.

Laboratory Experiments.—To study the residual behavior of lindane, 1000 grams of Miami silt loam were placed into 1-gallon wide mouth jars and measured amounts of lindane in an acetone solution were pipetted onto the soil at a rate of 10 parts per million. Thereafter the soil was mixed by rolling horizontally on a specially designed apparatus (fig. 1) for one-half hour. The acetone was evaporated by passing a slight air stream over the soil. After the soil was placed on a sheet of paper (30″×30″) an additional thorough mixing was employed by rolling the soil in different directions (fig. 2). Aliquots were then taken for analysis and for water determination of the initial sample. The treated soil was then placed into shallow pyrex dishes (6½″×10½″×2″). Two replicates were held at 46° C. for a period of 56 days, and distilled water was used to keep the soil wet during the time of the ex-

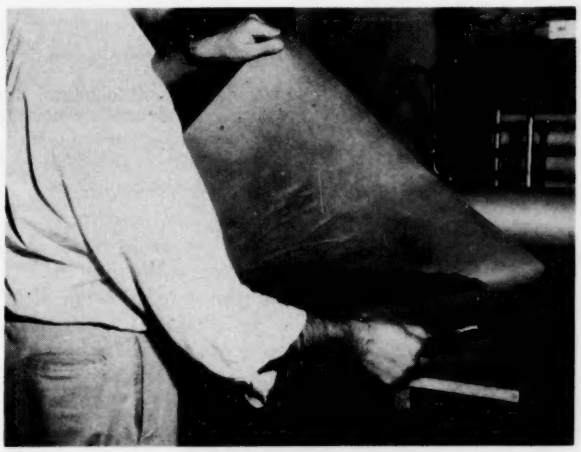

FIG. 2.—Mixing of screened soil on paper.

periment. Samples were taken initially, and at 14, 28 and 56 days thereafter.

When aldrin was used, the treatment of a Miami silt loam was done in the same way as described for lindane. After thorough mixing, two to four replicates in shallow dishes were held at either 7° C., 26° C. or 46° C. over a period of 84 days and kept wet with distilled water during the time of the experiment. Samples were taken initially, and at 14, 28, 56 and 84 days thereafter.

ANALYTICAL METHODS.—*Colorimetric Analysis.*—When field samples were extracted, the entire samples were screened and then thoroughly mixed by rolling on a sheet of paper. When laboratory samples were analyzed, the soil in the dish was screened and thoroughly mixed. Aliquots were then taken for extraction. Besides this, 50 or 100 grams of soil were dried for 24 hours at 46° C. to determine the dry weight of the soil. Lindane was analyzed according to the Schechter-Hornstein method (Schechter & Hornstein 1952). A change of the method (Lichtenstein *et al.* 1956), eliminated a special extraction procedure and permitted the determination of lindane directly in soils and crops.

Soils treated with aldrin were handled in the following way: when field samples were analyzed, 400 grams of field moist soil were placed into a 2-quart wide mouth Mason jar together with 200 grams of anhydrous sodium sulfate. When laboratory samples were analyzed, 50 to 200 gram aliquots of soil—depending on the amount of insecticide present—and 50 to 100 grams anhydrous sodium sulfate were taken. A mixture of redistilled hexane and isopropanol (2 to 1) was used as an extraction solvent. Two milliliters of solvent per 1 gram of wet soil were taken and a 1-hour head to end tumbling was applied. After tumbling, the solvent was decanted through filter paper and the recovered volume was recorded to be used for calculating the results. After that, the isopropanol was removed from the soil extract by washing twice with water and then three times with a saturated solution of sodium chloride. The alcohol-free phase was then dried over anhydrous sodium sulfate.

Since the extracts were analyzed for aldrin and dieldrin, clean up as well as separation of the two insecticides was done by using a 6″ florisil (60×100 mesh) column (20 mm. diameter). After the extract had been added to the column, aldrin was eluted by adding 200 ml. of redistilled hexane. The dieldrin remained on the florisil and could be washed from the column with 700 ml. of a mixture of redistilled hexane and benzene (4 to 1). The first 150 ml. collected contained colored substances, but no dieldrin. Therefore, only the remaining 550 ml. were used for the dieldrin analysis. The hexane-benzene solution was then evaporated to dryness and the dieldrin taken up in 2 ml. of redistilled hexane. Aldrin and dieldrin fractions were analyzed according to the method of O'Donnell *et al.* (1954, 1955). Known amounts of aldrin added to soils were recovered to an extent of 92 to 98% and known amounts of dieldrin added to soils were recovered to an extent of 91 to 94%. Each analysis was run in duplicate, using a soil blank for the determination of apparent insecticide.

Bioassay.—For bioassay a direct feeding method was employed (Edwards *et al.* 1957), using *Drosophila melanogaster* Meig. as a test insect. The flies were exposed to the soils directly, thus eliminating a special extraction procedure, which is time-consuming and influences, to some extent, the accuracy of the analysis. All aldrin-treated soils, though containing at the time of the analysis aldrin and dieldrin, were measured against an aldrin standard.

RESULTS AND DISCUSSION.—*Breakdown of Lindane in Soils.*—When analyses of lindane treated soil were conducted, it was found that the figures for residue data obtained by bioassay were always smaller than those obtained by chemical analyses. This might be explained by the fact that certain breakdown products of lindane are still detected by the Schechter-Hornstein method. But if those degradation products are nontoxic, they will not be detected by bioassay procedures.

When a Miami silt loam was treated in the laboratory with lindane at a rate of 10 p.p.m., good agreement was obtained initially between the chemical and bioassay estimates of the amount of insecticide present (fig. 3, table 1). However, 2 weeks later 89% of the initially applied lindane was recovered by chemical analysis and only 60% by bioassay. This seems to indicate that 40% of the originally applied toxicant had disappeared during those first 2 weeks. But 29% (=89%−60%) of the recovered lindane by chemical analysis most probably represents a nontoxic breakdown product which was still detected by the Schechter-Hornstein method.

Four and eight weeks after treatment, the recoveries obtained by bioassay still amounted to 66% of the esti-

Table 1.—Recovery of lindane from Miami silt loam and sandy loam soils as tested by chemical analyses and bioassays.

YEARS AFTER TREATMENT	TREATMENT	LOAM SOIL TYPE	CHEMICAL ASSAY (P.P.M.)	BIOASSAY (P.P.M.)	BIOASSAY / CHEMICAL ASSAY ×100
Laboratory tests	*p.p.m.*				
0	10	Miami silt	9.89±0.31	9.45±0.82	96
1/24	10	Miami silt	8.92±0.38	6.01±0.45	67
1/12	10	Miami silt	5.38±0.19	3.65±0.10	68
1/6	10	Miami silt	4.58±0.11	2.83±0.13	62
Field tests	*lb./6″ acre*				
3	10	Miami silt	0.56±0.02	0.40±0.05	70
3	100	Miami silt	11.3±0.20	6.79±0.15	60
3	10	sandy	0.81±0.09	0.59±0.03	73
3	100	sandy	16.05±0.40	9.90±0.42	62
3.5	10	sandy	0.63±0.04	0.29±0.01	46
3.5	100	sandy	12.15±0.20	5.63±0.08	46

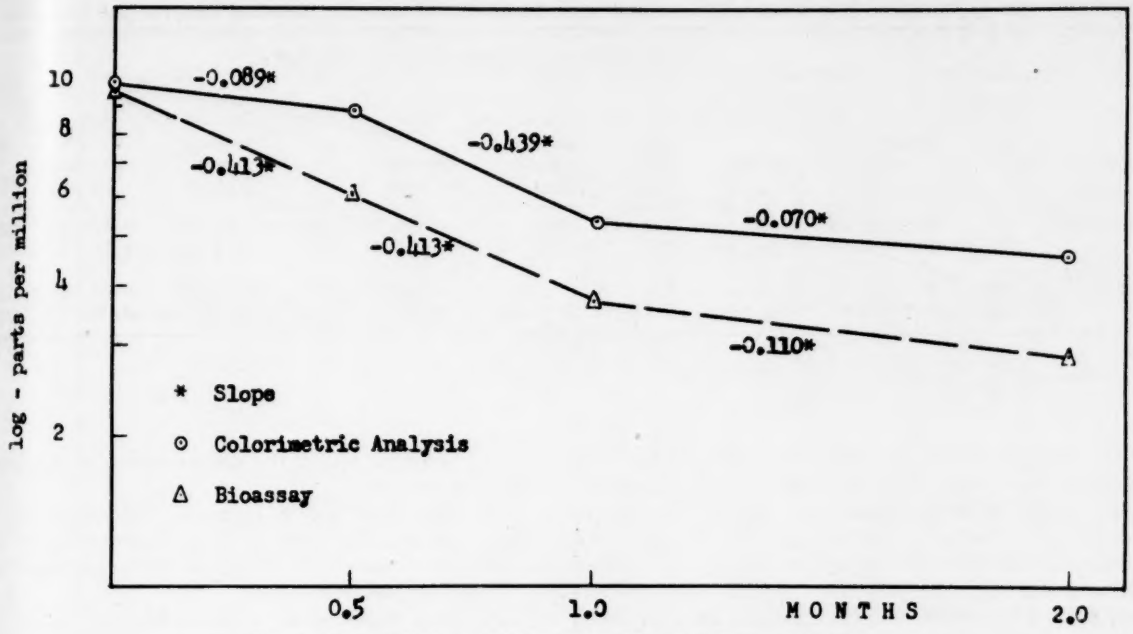

FIG. 3.—Loss of lindane residues from a Miami silt loam at 46° C.

mates secured by chemical analyses. The slopes of the depletion curves obtained by chemical assays and bioassays are different during the first 2 weeks, but are very similar later on (fig. 3). Therefore, the breakdown of lindane into a nontoxic compound probably occurs during the first 2 weeks after treatment. From that time on the rate of disappearance of lindane as well as the nontoxic degradation product seems to be similar for 42 days.

Under field conditions, the same ratio between bioassay and chemical results was found, when samples of lindane containing soils were collected and analyzed, 3 years after treatment (table 1). There was some variation in the amount of insecticide recovered from the various lindane-treated field plots. In the Miami silt loam, 9.2% to 18.4% of the originally applied lindane was recovered and 15.2% to 30.6% of the applied lindane was found in the sandy loam. But in all field plots investigated, bioassay recoveries ranged from 60% to 73% of the recoveries obtained by chemical analyses.

At a somewhat later date—3½ years after treatment—only 46% of the amounts found by chemical analyses were recovered by bioassay. This might indicate that during the additional summer season the lindane broke down further, but the nontoxic breakdown product(s) was more stable and caused the bigger discrepancy between the chemical and bioassay results.

The application rate of 100 pounds per 6-inch acre to a sandy loam represents a 52.5 p.p.m. treatment. The analytical results obtained 3½ years after treatment with lindane at a rate of 100 pounds per 6-inch acre might be expressed in per cent recovery: 23.2% of the originally applied insecticide was recovered by chemical analysis and 10.7% by bioassay. During the 3½ years, in which the insecticide was in the soil, 89.3% (=100%−10.7%) of the applied toxicant had disappeared. However, 12.5% (=23.2%−10.7%) of the applied insecticide still persisted after 3½ years in the form of a nontoxic compound which was still detected by the Schechter-Hornstein method.

For all practical purposes the bioassay results seem to give the more realistic assessment of the insecticidal residues.

CONVERSION OF ALDRIN TO DIELDRIN IN SOILS.—*Field Experiments.*—The disappearance of aldrin from soils has been reported (Lichtenstein & Schulz 1958) to be relatively fast, the rate of decline dependent, to a large extent, on the soil type in which the aldrin is present. However, depletion curves based on the specific colorimetric method for aldrin, do not give a true picture of toxic residues present. Contrary to the findings with lindane, residue estimates obtained by bioassay of aldrin treated soils were considerably larger than the ones obtained by chemical analyses (table 2). Two years after treatment, 1.7 to 5.22 times more toxicant was found by bioassay than by chemical analysis, 1.40 to 13.40 times more after 3 years and 1.26 to 7.3 times more 4 years after treatment. This seemed to indicate that part of the originally applied aldrin had been converted into another toxicant which was not detected by the phenylazide method for aldrin, but showed up in the bioassay procedure. This toxicant proved to be dieldrin (table 3).

Soils which had been treated at a rate of 20 pounds per 6-inch acre showed the greatest difference between the bioassay results and the chemical ones, 3 years after treatment. Apparently an increase in the amount of dieldrin occurred during the first 3 years after treatment, followed by a decrease.

Standard curves prepared with aldrin or dieldrin for the determination of LD_{50} values for *Drosophila* showed that aldrin was 1.8 times more toxic than dieldrin, when *Drosophila* flies were used in a direct exposure method. Since all the aldrin treated soils were measured against an aldrin standard only, the results obtained by bioassay are actually higher than presented in table 2.

Table 2.—Aldrin recoveries from Miami silt loam and sandy loam soils as tested by chemical analyses and bioassays.

Loam Soil Type	Amount Applied Lb./6″ A.	Years Between Treatment and Sampling: 2 Chemical Assay (p.p.m.)	2 Bioassay (p.p.m.)	2 $\frac{B}{C}$[a]	3 Chemical Assay (p.p.m.)	3 Bioassay (p.p.m.)	3 $\frac{B}{C}$[a]	4 Chemical Assay (p.p.m.)	4 Bioassay (p.p.m.)	4 $\frac{B}{C}$[a]
Miami silt	20	0.69±0.03	1.89±0.14	274	0.37±0.04	1.50±0.10	406	0.14±0.01	0.47±0.02	336
	200	16.50±0.20	28.00±2.0	170	7.92±0.21	11.10±0.39	140	3.69±0.32	4.64±0.04	126
Sandy	20	0.24±0.03	1.25±0.11	522	0.11±0.01	1.53±0.08	1340	0.08±0.01	0.59±0.02	730
	200	22.25±0.30	66.00±0.55	293	11.35±0.25	37.40±1.40	332	5.65±0.13	13.00±1.6	230

[a] $\frac{B}{C}$ = Ratio $\frac{\text{Bioassay}}{\text{Chemical assay}} \times 100$.

The first soil samples analyzed for dieldrin, in addition to aldrin, were collected in fall 1956, 2½ years after treatment. At that time 5.85 times more dieldrin than aldrin was found in the loam plot treated with 20 pounds of aldrin per acre. However, in the plot treated at a rate of 200 pounds per acre, the amount of dieldrin recovered was only 81% of the aldrin found in the loam. In a muck soil the amount of dieldrin recovered amounted to 190% (2 lbs./acre treatment), 116% (20 lbs./acre treatment) and 30% (200 lbs./acre treatment) of the aldrin found in the soil.

Four years after treatment with aldrin (20 pounds per 6-inch acre), 12.6 times more dieldrin than aldrin was recovered from a sandy loam, six times more from a Miami silt loam and 1.79 times more from a muck soil (table 2). Plots which had been treated with 200 pounds per 6-inch acre contained only 2.70 (sandy loam) and 1.37 (Miami silt loam) times more dieldrin than aldrin. In a muck soil the amount of dieldrin recovered amounted to 31% of the amount of aldrin found. This percentage is the same as established 1½ years earlier for the same muck plot.

As the results obtained by bioassay already indicated, it has been shown that relatively more aldrin was converted into dieldrin in those soil plots in which the insecticide was less concentrated. It has been found, too, that aldrin, lindane and DDT persisted longer in those soils, where they were most concentrated (Lichtenstein & Schulz 1958). Apparently the reactions going on within the soil and being responsible for the disappearance and breakdown of those insecticides investigated, are dependent to some extent on the concentration of the insecticides in the soil.

The amount of aldrin converted into dieldrin was greatest in a sandy loam, less in a Miami silt loam and smallest in a muck soil, though all three soil types had received identical treatments.

Laboratory Experiments.—When a Miami silt loam was treated with aldrin at a rate of 10 p.p.m., part of the aldrin was found to be converted into dieldrin during the weeks following treatment (table 4 and fig. 4). While the decline of aldrin progressed with time, though to a smaller extent at lower temperatures, the formation of dieldrin increased and reached its peak 56 days after treatment, in soils held at either 26° C. (1.49 p.p.m. of dieldrin recovered) or 46° C. (1.05 p.p.m. of dieldrin recovered). Eighty-four days after treatment less than one-half the amount of dieldrin found 4 weeks earlier was recovered from soils held at 26° C. (0.66 p.p.m. dieldrin) and 46° C. (0.40 p.p.m. dieldrin).

In aldrin treated soils held at 7° C., between 3 to 4% of the total amount of insecticides recovered were found to be dieldrin and no change in the formation of dieldrin

Table 3.—Recovery (in p.p.m.) of aldrin and dieldrin from three soil types 4 years after treatment with aldrin (chemical analyses).

Soil Type	Aldrin Applied (Lb./6″ A.)	Aldrin	Dieldrin	D/A[a]
Muck	2	0.07±0.02	0.21±0.02	3.00
Muck	20	1.43±0.06	2.56±0.08	1.79
Muck	200	62.80±0.43	19.30±0.60	0.31
Miami silt loam	2	0.00	0.17±0.01	
Miami silt loam	20	0.14±0.01	0.84±0.03	6.00
Miami silt loam	200	3.69±0.32	5.02±0.18	1.37
Sandy loam	2	0.00	0.09±0.01	
Sandy loam	20	0.08±0.01	1.01±0.04	12.60
Sandy loam	200	5.65±0.13	15.30±0.60	2.70

[a] $\frac{D}{A}$ = Ratio: $\frac{\text{Dieldrin recovery}}{\text{Aldrin recovery}}$.

Table 4.—Recovery in p.p.m. of aldrin and dieldrin from a Miami silt loam (at 7° C., 26° C., and 46° C.), treated with aldrin only at a rate of 10 p.p.m. (Chemical analyses).

Days After Treatment	Aldrin	Dieldrin	A[a] and D[b]	Per Cent of Total: A[a]	D[b]
		At 7° C.			
14	8.65±0.39	0.00	8.65	100.0	0.0
28	8.77±0.33	0.45±0.06	9.22	95.0	5.0
56	8.08±0.15	0.22±0.01	8.30	96.0	4.0
84	7.27±0.26	0.13±0.03	7.40	98.0	3.0
		At 26° C.			
14	8.15±0.15	0.33±0.02	8.48	96.0	4.0
28	7.59±0.14	0.62±0.05	8.21	92.0	8.0
56	5.31±0.14	1.49±0.01	6.80	78.0	22.0
84	3.40±0.22	0.66±0.02	4.07	84.0	16.0
		At 46° C.			
14	5.93±0.48	0.51±0.06	6.44	92.0	8.0
28	3.39±0.06	0.59±0.10	3.98	85.0	15.0
56	1.34±0.05	1.05±0.05	2.39	56.0	44.0
84	0.74±0.04	0.40±0.03	1.14	65.0	35.0

[a] A = Aldrin.
[b] D = Dieldrin.

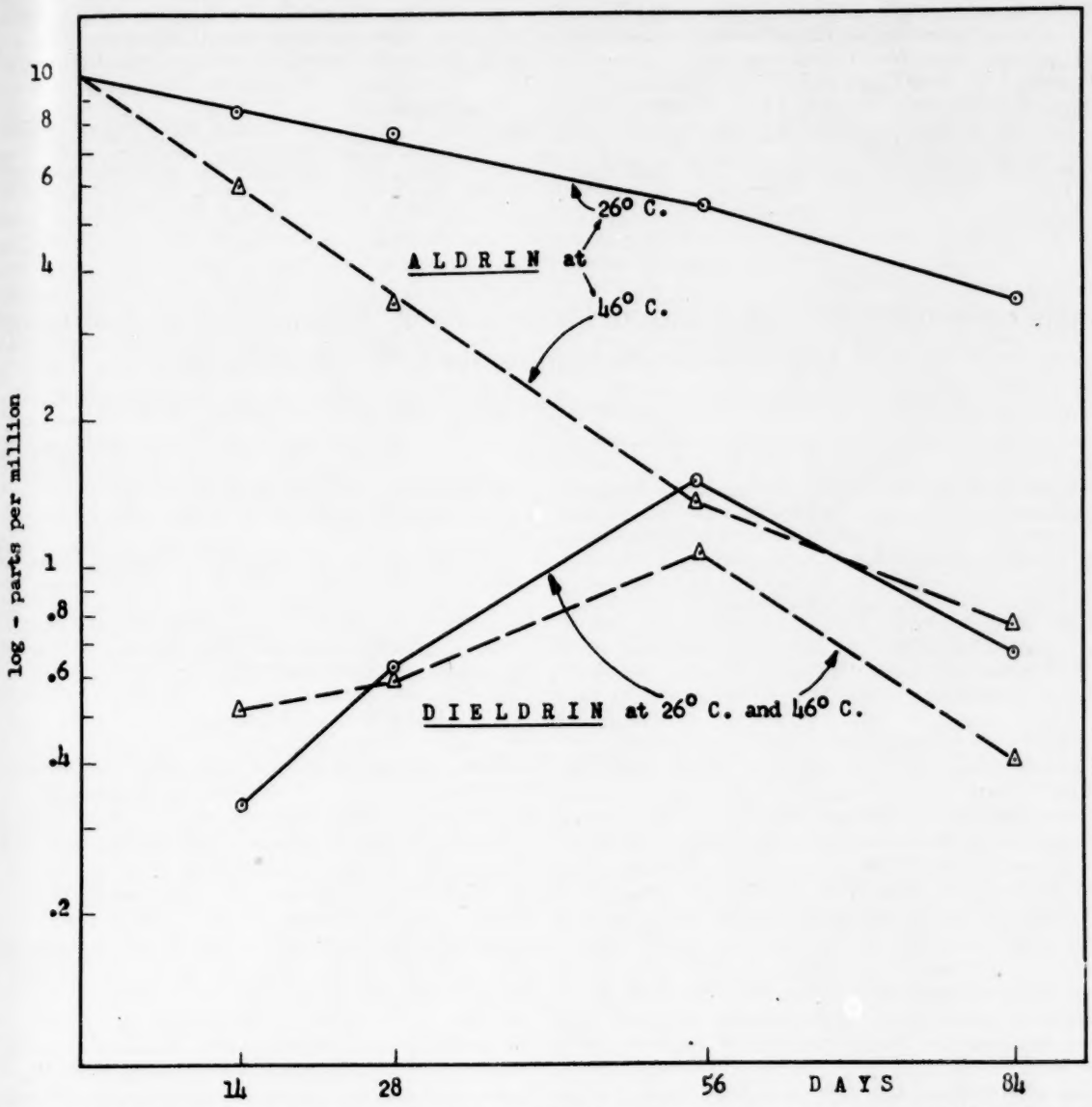

FIG. 4.—Recoveries of aldrin and dieldrin residues from a Miami silt loam at 26° C. and 46° C. Treatment: Aldrin, 10 p.p.m.

was noticed as time progressed. However, at a temperature of 26° C., the amount of dieldrin recovered after 56 days was 22% of the total insecticide recovered at that time and amounted to 44% of the total amount of aldrin and dieldrin found in soils which were held at 46° C.

All these data (field and laboratory experiments) indicate that some of the aldrin in soils is converted into dieldrin, the amount of dieldrin formed being dependent to some extent on the soil type and the temperature. A gradual accumulation of dieldrin occurs, followed by a decrease after the peak of the dieldrin concentration has been reached.

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Persistence of Some Chlorinated Hydrocarbon Insecticides as Influenced by Soil Types, Rate of Application and Temperature[1,2]

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ABSTRACT

Field and laboratory experiments were conducted to determine the influence of soil type, rate of application, and temperature on the persistence of some chlorinated hydrocarbons in soils. Three and a half years after treatment 1.43 times more DDT, 4.25 times more aldrin and 8.45 times more lindane were recovered from a muck soil than from comparably treated Miami silt loam. A field plot (Miami silt loam) treated at a rate of 100 pounds per 6-inch acre contained—3½ years after treatment—2.03 times more DDT than the plot treated at a rate of 10 pounds per 6-inch acre. The comparable figure for lindane was found to be 3.50. Under laboratory conditions, figures comparable to field results were obtained when soils were incubated at 46° C. for a period of 56 days.

Soil temperatures were found to be an important factor in the persistence of insecticides in soils. No insecticide loss was found in frozen soils. At a temperature of 6° C. aldrin and heptachlor (16% to 27%) were lost during a period of 56 days and only 2% to 14% of the initial insecticides were found after 56 days, when held at a temperature of 46° C.

Chlorinated hydrocarbon insecticides, used extensively during the last decade, have presented a major residue problem. Insecticides from sprayed crops and from direct soil applications have been found to persist or even to accumulate in soils (Chisholm *et al.* 1951, Fleming *et al.* 1951, Ginsburg & Reed 1954, Ginsburg 1955, Lichtenstein 1956 and 1957, Kiigemagi *et al.* 1958). Such accumulation might be of value in the control of certain soil insects, but may have a detrimental effect on subsequent crops. Not only may root systems be affected by those residues, but some may be translocated into the crop tissue and cause off flavor (Simkover & Shenefelt 1951 and 1952, Stone *et al.* 1953, Fleming & Maines 1953, Terriere & Ingalsbe 1953, Allen *et al.* 1954, Boswell *et al.* 1955). Moreover, a soil, being a complex of living and dead material supporting plant growth, might be affected by pesticide chemicals and their breakdown products. The persistence of residues in soils is dependent on various factors such as the insecticide itself, soil types and their biological and physical properties, as well as climatic conditions (Young 1956, Bollen 1958). Field and laboratory studies conducted to determine the effect of soil types, rate of application and temperature on the persistence of DDT, lindane, aldrin, and heptachlor are reported in this paper.

PROCEDURE.—*Field Studies.*—In May, 1954, a Miami silt loam and a muck soil were treated with DDT at 10 and 100 pounds per acre, lindane at 10 and 100 pounds per acre and aldrin at 20 and 200 pounds per acre. The insecticides were applied as described by Lichtenstein & Schulz (1958). Soil samples were collected initially and 6, 12, 18, 24, 30, 36, and 42 months thereafter. Sampling was done with a soil auger and 40 cores (¾ inch diameter 6 inches long) were collected from each plot. The area of the experimental plots was reduced to 21′×42′ for sampling, leaving a 4′ strip between the boundaries of the treated plot and the sampling area.

Laboratory Experiments.—To study the effect of soil types on the persistence of aldrin, 900 to 1100 gm. of muck, Miami silt loam or Plainfield sand each were placed into 1-gallon wide mouth jars and a measured amount of recrystallized aldrin in an acetone solution was pipetted onto the soil at a rate of 200 pounds per 6-inch acre. Thereafter the soil was mixed according to the procedure described by Lichtenstein & Schulz (1958). After aliquots had been taken for extraction and for water determination of the initial sample, the soil was placed into shallow pyrex dishes (6½″×10½″×2″). Two to four replicates were then held at 26° C. The soils were watered with distilled water during the time of the experiment.

When the effect of temperature was tested, soils were treated in the same way with aldrin or heptachlor at a rate of 20 pounds per 6-inch acre. After thorough mixing, two to four replicates in shallow pyrex dishes were held at either 7° C., 26° C., or 46° C. and kept wet with distilled water during the time of the experiment. Samples

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were taken initially and 3, 7, 14, 28 and 56 days thereafter. Before each extraction, the soil in the dish was thoroughly mixed and aliquots were then taken for extraction and water determination.

Analytical Methods.—Prior to the extraction of the insecticides field and laboratory samples were handled as described by Lichtenstein & Schulz (1958). The extraction solvents used were a mixture of benzene-isopropanol (2 to 1) for DDT, a mixture of redistilled hexane and isopropanol (2 to 1) for aldrin and a mixture of colorimetric pentane and acetone (4 to 1) for heptachlor. Two milliliters of solvent were used per gram of wet soil and a 1-hour head to end tumbling was applied. After tumbling the supernatant liquid was decanted through filter paper and the recovery volume was recorded, to be used for calculating the results. When heptachlor was extracted, the jars containing soil and solvent were placed—after tumbling—for one-half hour, into a refrigerator, in order to minimize the evaporation of pentane while filtering.

The isopropanol or the acetone was removed from the soil extracts by washing first with water and then with a saturated solution of sodium chloride. The acetone or alcohol free phase was then dried over anhydrous sodium sulfate.

DDT extracts were cleaned up by passing through a column (6″×1″) of aluminum oxide and analyzed according to the Schechter-Haller method (Schechter & Haller 1945). Aldrin extracts were cleaned up by passing through a column (8″×1″) of Attasol-Celite and analyzed according to the Phenylazide method (O'Donnell *et al.* 1954).

Heptachlor extracts were cleaned up by using "Nuchar" activated carbon (C 190-n) (Lichtenstein & Schulz 1958) and analyzed according to the Polen-Silverman method (Polen & Silverman 1952, Ordas *et al.* 1956).

Lindane was analyzed according to the Schechter-Hornstein method (Schechter & Hornstein 1952). A change of the method (Lichtenstein *et al.* 1956), eliminated a special extraction procedure and permitted the determination of lindane directly in soils and crops.

Known amounts of insecticides added to soils were recovered to an extent of 92 to 98%.

Each analysis was run in duplicate using a soil blank for the determination of apparent insecticide. In addition, known amounts of insecticide were added to insecticide-free soil samples. This permitted a check of the analytical procedure for each analysis done. The unknowns, after the values for apparent insecticide had been subtracted, were calculated on the basis of the values obtained for the known amounts. Results were expressed in parts per million, based on the dry weight of the soil under investigation.

RESULTS AND DISCUSSION.—1. *Insecticide persistence as influenced by soil types*, was tested under field and laboratory conditions. Figure 1 illustrates the rate of loss of DDT, lindane and aldrin under field conditions in a muck soil (Organic matter content: 49.0%) and a Miami silt loam (Organic matter content: 3.8%) over a period of 3½ years. All three insecticides tested were found to be more persistent in a muck than in a Miami silt loam. Thirty-three per cent of the originally applied DDT and lindane were recovered after 3½ years from a muck soil and 4.7% of the originally applied aldrin. In a Miami silt loam, 22.7% of the DDT, 3.9% of the lindane and 1.1% of the aldrin were still found to be present.

The rate of loss was found to be highest during the first 6 months after application, though less in a muck soil than in a Miami silt loam (table 1). For instance, one-half of the amount of the aldrin applied had disappeared 3.75 months after application to a muck soil and 2.4 months after application to a Miami silt loam. Thereafter, half the amount found after the first 6 months had disappeared after 13 months from a muck soil and after 9.7 months from a Miami silt loam.

DDT was found to be the most persistent of the three insecticides investigated, and the difference in the rate of loss as influenced by a muck and a loam soil was the least noticeable. The depletion curves obtained for DDT in those two soil types were nearly parallel after the initial loss during the first summer seaon. The amount of DDT recovered from a muck soil was 1.4 times more than recovered from a Miami silt loam, 6 months after soil treatment. The same ratio was obtained, when both soil types were analyzed for DDT during the following 3 years (table 2).

Of the insecticides studied, the persistence of lindane was most affected by soil types, and the recovery ratios between muck and loam became larger as time progressed. Six months after application of lindane, 1.84 times more of the insecticide was recovered from a muck than from a loam soil. However, three years later, the recovery ratio was found to be 8.45.

The persistence of aldrin was tested under laboratory conditions (at 26° C.), after having been applied to a muck, Miami silt loam and Plainfield sand (Organic matter content: 0.8%). The decline of aldrin in a muck soil followed a straight line (Slope −0.029), when residue values were plotted as a log function against days (fig. 2). However, depletion curves obtained for aldrin in a Miami silt loam as well as in a Plainfield sand showed different negative slopes, with the greatest decline during the first month. Fifty-six days after treatment, 87.5% of the initially recovered aldrin was still found to be present in the muck soil, 68.9% in a Miami silt loam and 54.5% in the Plainfield sand. The amount of aldrin, recovered 2 months after soil treatment from a muck soil was 1.27 times larger than the amount recovered from a loam soil. Under field conditions and 6 months after insecticide application, 1.94 times more aldrin was recovered from a muck soil than from a Miami silt loam.

However, the residue picture presented here for lindane and aldrin does not seem to be a realistic one. Edwards *et al.* (1957) reported that aldrin had been converted to dieldrin within soils. Later on, other workers (Bollen *et al.* 1958, Gannon & Bigger 1958) confirmed the same, and Lichtenstein & Schulz (1958) reported that 4 years after soil treatment with aldrin at a rate of 20 pounds per 6-inch acre, six times more dieldrin than aldrin was recovered from a Miami silt loam, and 12.6 times more from a sandy loam. Therefore, the actual amount of toxicants present in aldrin-treated soils is higher than the residue figures presented here for aldrin. Moreover, lindane was found (Lichtenstein & Schulz 1958) to break down within soils to a nontoxic compound, which was still detected by the colorimetric analysis for lindane, but

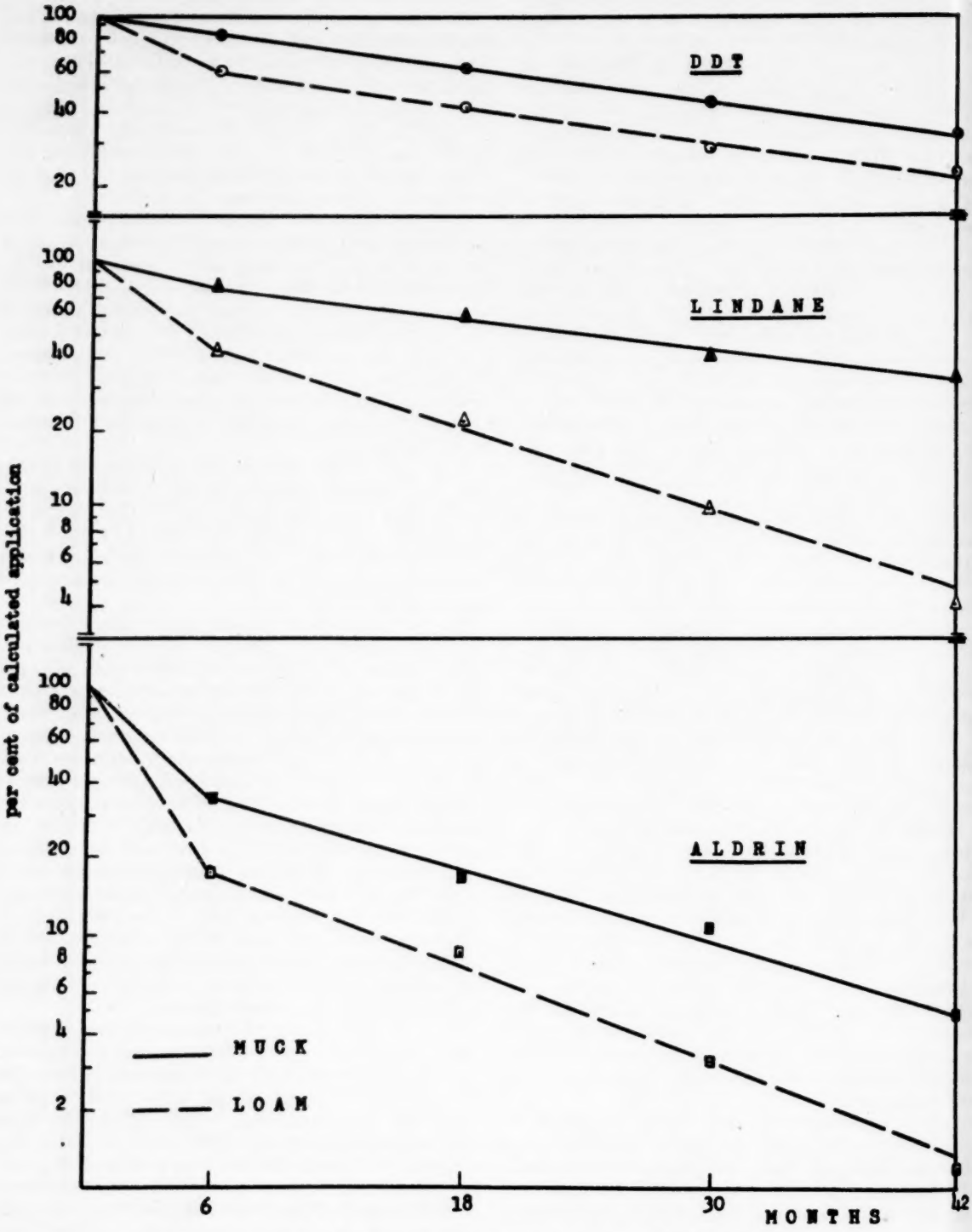

FIG. 1.—Rate of loss of insecticide residues from a muck and a Miami silt loam. Application rates: DDT and lindane—10 lb/6″ acre; Aldrin—20 lb/6″ acre.

Table 1.—Half life ($T_{\frac{1}{2}}$) of DDT, lindane and aldrin in a muck soil and a Miami silt loam under field conditions.

Insecticide Applied (Lb./6″ A)	$T_{\frac{1}{2}}$ in Months[a]			
	Muck		Loam	
	First Half Year	Following Three Years	First Half Year	Following Three Years
DDT 10	20	27.3	7.75	24.6
Lindane 10	15.5	28.8	4.75	11.1
Aldrin 20	3.75	13.0	2.40	9.7

[a] $T_{\frac{1}{2}} = \frac{2.303 \times \log 2}{K}$; K = slope × 2.303.

Table 2.—Comparison of insecticide persistence in muck and loam soils (Field experiments).[a]

Months After Treatment	Muck/Loam[b]		
	DDT	Lindane	Aldrin
6	1.40	1.84	1.94
18	1.46	2.62	2.00
30	1.55	4.20	3.45
36	1.43	8.45	4.25

[a] Application rates: DDT and lindane 10 lb./6″-acre Aldrin 20 lb./6″-acre.
[b] Per cent recovery of initial application dose from muck / Per cent recovery of initial application dose from loam

not by bioassay. Three and one-half years after treatment, the quantity estimated by bioassay amounted to 46% of that obtained by chemical analysis. Contrary to aldrin, the actual amounts of toxicants present in lindane-treated soil are smaller than the residue figures obtained by chemical analysis.

2. *The effect of insecticide concentration on loss of DDT, lindane and aldrin from a Miami silt loam* is summarized in figure 3 and table 3. Under field conditions, 2.03 times more DDT was recovered after 3½ years from the plot that had been treated at the higher rate of application than from the one treated at the lower rate. The respective figures for lindane were found to be 3.50 and for aldrin 2.27. But, even 6 months after soil treatment, the discrepancy in per cent recoveries of insecticides from soils, having been treated at high and low rates of application, was obvious, though to a somewhat smaller extent than 3 years later. During the first 6 months following insecticide applications, half life values ($T_{\frac{1}{2}}$) obtained for DDT—applied at the low (10 pounds per 6-inch acre) and high (100 pounds per 6-inch acre) dosages—were 7.75 and 39.7 months, respectively; for lindane, 4.75 and 6.98 months; and for aldrin, 2.40 and 3.10 months.

When a Miami silt loam, treated with aldrin at the rates of 20 pounds and 200 pounds per 6-inch acre was incubated at 7° C., 26° C. and 46° C., only the soil being held at 46° C. showed a difference in recovery—56 days after treatment—comparable to the field tests (table 3b). At this temperature, 2.22 times more aldrin was recovered from the loam treated at a rate of 200 pounds per 6-inch acre as compared with the one that had been treated at a rate of 20 pounds per 6-inch acre. From these figures it might be assumed that the disappearance of the insecticides investigated is dependent on the rate of application. When the concentration of the insecticides in the soil is lower, they seem to disappear faster.

3. *The effect of various temperatures on the rate of insecticide loss from soils* is illustrated in figures 4 and 5 and in table 4. In all the experiments conducted the disappearance of aldrin as well as heptachlor was comparatively rapid when the soils were incubated at a temperature of 46° C. But even in soils kept at 7° C., some loss of aldrin and heptachlor was noticeable. In other experiments, it was shown that no loss occurred when treated soils were frozen for a period of 6 months.

In a Miami silt loam the persistence of aldrin and heptachlor was similar at 7° C. and 26° C. However, at 46° C. and 56 days after treatment, less heptachlor (1.8% of initially recovered) was found than aldrin (13.7% of initially recovered). In Plainfield sand, aldrin loss was more rapid at all three temperatures than in a treated loam.

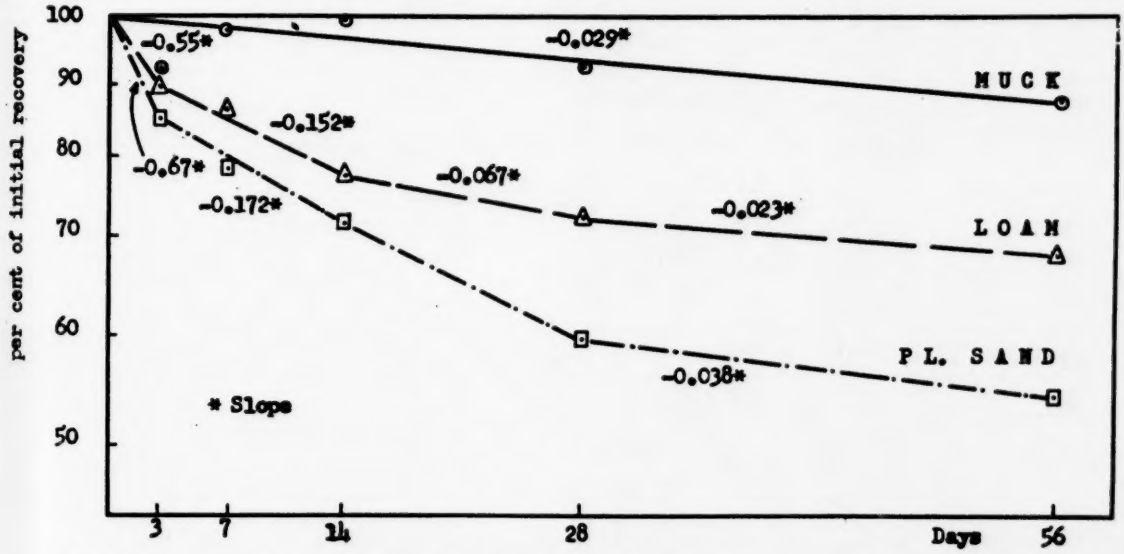

FIG. 2.—Loss of aldrin residues at 26° C. as affected by three soil types. Application rate: 200 lb./6″ acre.

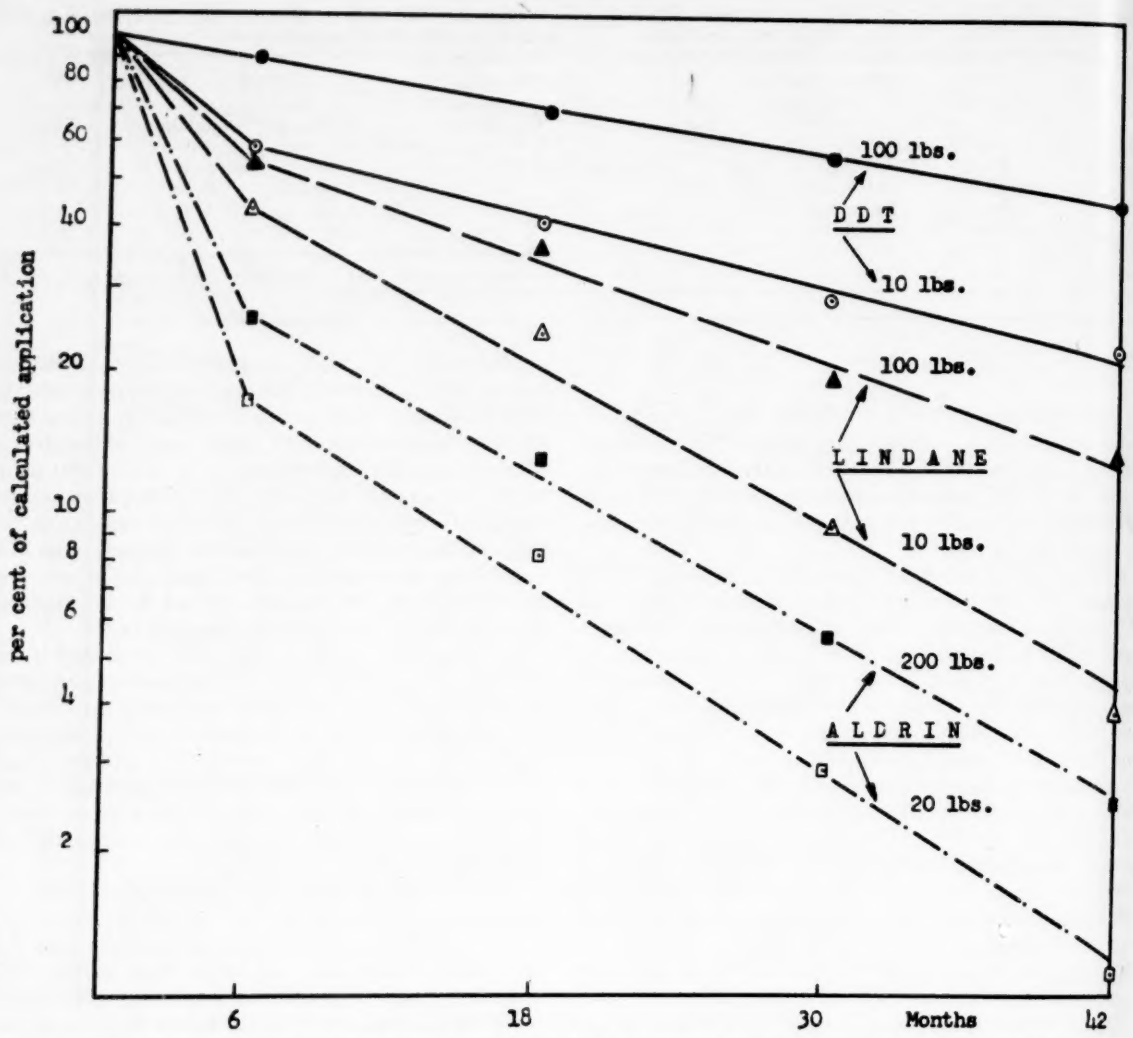

FIG. 3.—Effect of concentration on loss of DDT, lindane and aldrin from a Miami silt loam. Application rates: DDT and lindane—10 lb. and 100 lb/6″ acre; Aldrin—20 lb. and 200 lb./6″ acre.

Table 3.—Effect of concentration on rate of insecticide loss from a Miami slit loam.

Insecticide	Pounds/6″ Acre Applied				High/Low Concentration[a]
	10	20	100	200	
(A) *Residues under field conditions, 3½ years after application (in per cent of calculated application)*					
DDT	22.7±2.15	—	46.0±0.3	—	2.03
Lindane	3.9±0.11	—	13.7±0.37	—	3.50
Aldrin	—	1.12±0.05	—	2.55±0.42	2.27
(B) *Residues of aldrin under laboratory conditions, 56 days after treatment (in per cent of initial recovery)*					
Incubation Temperature					
6° C.		83.8±1.75		84.4±1.85	1.01
26° C.		55.7±1.50		68.9±2.65	1.23
46° C.		13.7±0.5		30.4±5.05	2.22

[a] Per cent recovery from high insecticide application rate / Per cent recovery from low insecticide application rate

Table 4.—Effect of temperature on the rate of insecticide loss from a Miami silt loam and a Plainfield sand.[a]

Incubation Temperature (° C.)	Aldrin	Heptachlor
	Miami silt loam (20 lb./6″ acre)	
6	83.8±1.75	73.2±4.5
26	55.7±1.50	51.0±2.3
46	13.7±0.50	1.8±0.3
	Plainfield sand (100 lb./6″ acre)	
6	63.0±2.84	
26	38.0±1.62	
47	10.2±1.3	

[a] Expressed as per cent of initial recovery 56 days after treatment.

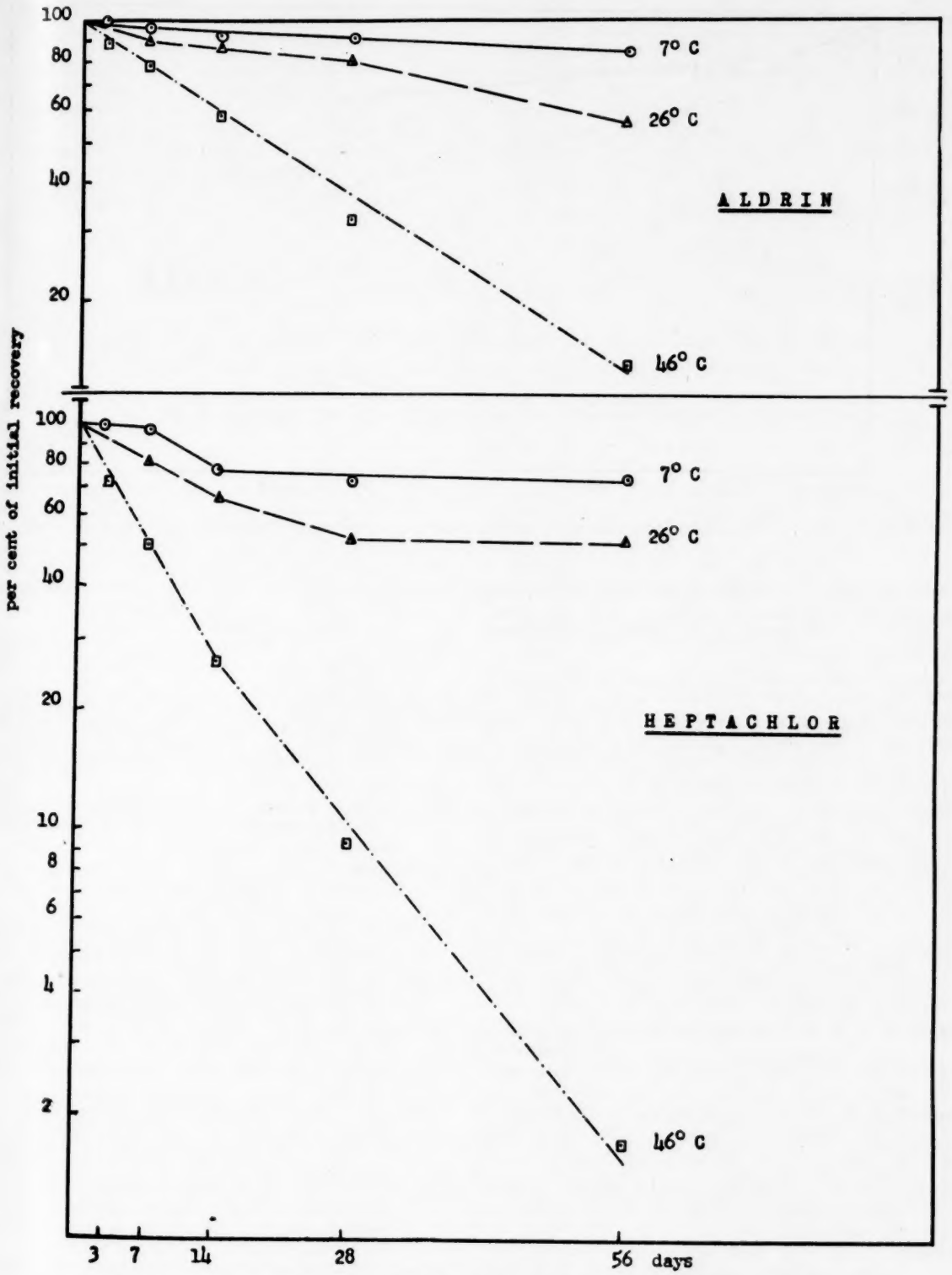

FIG. 4.—Loss of aldrin and heptachlor residues from a Miami silt loam as affected by temperature. Application rate: 20 lb./6″ acre.

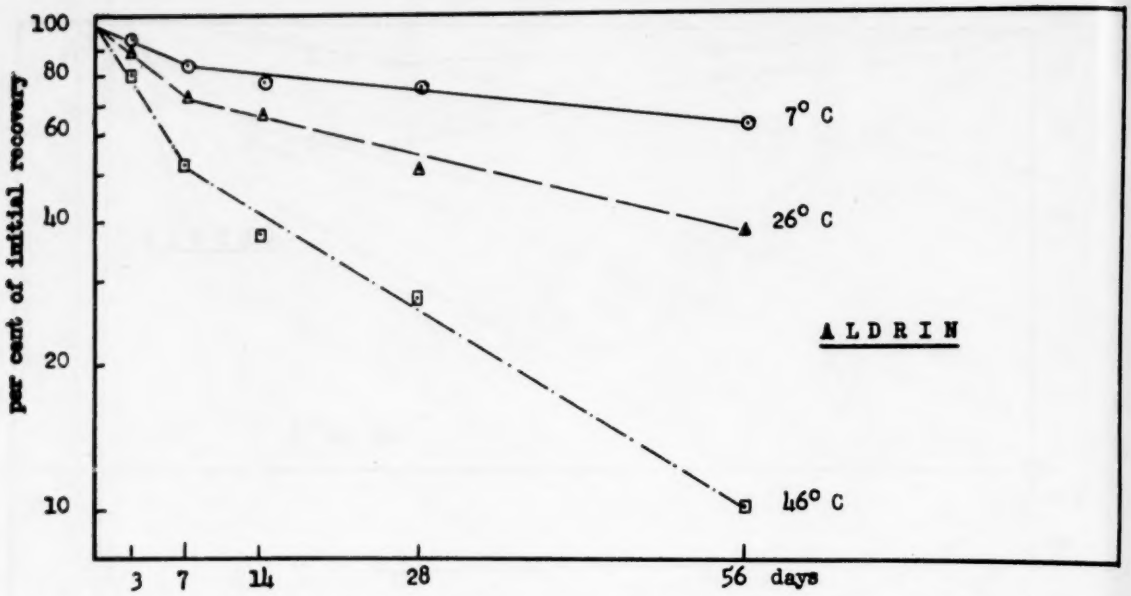

FIG. 5.—Loss of aldrin residues from a Plainfield sand as affected by temperature. Application rate: 100 lb./6″ acre.

Since heptachlor too has been found to be converted to its epoxide in soils (Gannon & Bigger 1958) the actual amount of toxicants present is probably somewhat higher than shown in figure 4.

Wollny (1883) reported that at an air temperature of 32° C., temperatures of 37° C. were recorded on the surface of the soil and 28° C. at a depth of 4 inches. On a sunny day in July, a temperature of 40° C. was recorded at a depth of 1½ inches on our loam plot. Since soil temperatures very often are relatively high in summer, the temperature of 46° C. used for incubation in our laboratory tests does not seem to be too much different from natural conditions encountered during the warm summer months.

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Effects of Grain Moisture Content on Residual Toxicity and Repellency of Malathion[1]

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ABSTRACT

Malathion applied at 2 p.p.m. to wheat of 13.5% moisture content caused 99% mortality of the rusty grain beetle, *Laemophloeus ferrugineus* (Steph.) at 8 months after treatment; the wheat was stored in airtight containers at 48° F. A dosage of 16 p.p.m. was required for comparable control in wheat of 15.5% moisture content but this dosage was ineffective in wheat of 18% moisture content at 5 months after treatment. Repellency tests in wheat of 12, 15 and 17% moisture content treated at 4 or 8 p.p.m., the test insects being placed in adjoining untreated wheat, showed that adults of *L. ferrugineus* but not of *Sitophilus granarius* (L.) were repelled from wheat of 12% moisture content treated at 4 p.p.m.; mortalities of both species ranged from 84.8 to 100.0% in treated wheats. Reproduction of *L. ferrugineus* was reduced more than that of *S. granarius* in unevenly treated wheat. There was no adverse effect on the milling or baking quality of wheat treated with malathion at 4 or 8 p.p.m.

Stored grain may be protected from insects by treating it with a residual insecticide. Such an insecticide must meet special requirements. First, it must have a low toxicity to humans and farm animals at the recommended dosages; second, it must not taint or discolor the product; third, it must be effective against grain-infesting insects during storage. These requirements limit the number and kind of insecticides that can be used in stored grain.

An insecticide used as a grain protectant may kill or repel adults and other exposed stages, and arrest oviposition. Repellency may be an advantage when grain has been uniformly treated; however, when it is not, insects may be repelled into pockets of untreated grain, where they may continue their depredations as long as they are not affected by contact with treated grain. The effectiveness of insecticides in stored grain depends, therefore, on their mode of action and residual toxicity.

Lindgren *et al.* (1954) reported that malathion applied at 2 p.p.m. to wheat containing 10% moisture was effective against the granary weevil, the rice weevil, and the lesser grain borer for 2 months; at 8 and 16 p.p.m., it remained effective for 6 to 7 months.

Wilbur (1952), and unpublished work at the Winnipeg laboratory, indicated that insecticides are less effective in grain of more than about 15% moisture content. Since heavy infestations often occur at this level, it is important to know how insecticidal effectiveness is influenced by moisture content.

This is a report on laboratory experiments on residual toxicity and repellency of malathion in wheat of different moisture contents.

MATERIALS AND METHODS.—The insecticide was supplied as 0.5% (w:w) malathion in a carrier of low-grade flour.[3] The rates recommended for dry wheat (less than 14.6% moisture content) and wheat of higher moisture content were 4 and 8 p.p.m. of toxicant, respectively.

For tests of toxicity, samples of No. 2 Northern wheat were conditioned with water to give moisture contents of 13.5, 15.5 and 18.0%. The moisture content of the wheat was measured with a Halross dielectric moisture meter.[4] Anderson (1956) reported that the standard error of estimate of this meter compared with the Brown-Duval method was 0.27% on 554 samples of winter wheat with moisture contents ranging from 12.2 to 17.6%. The samples were treated at 2, 4, 8 and 16 p.p.m. of malathion. They were stored for 2, 5 and 8 months in airtight containers to prevent changes in moisture content during aging of the deposits, and were kept at 48° F. to retard mold development.

After each aging period, the wheat was placed in pint jars with screen tops, each holding 150 gm. Three replicates were used for each dosage at each moisture content. The test insect was the rusty grain beetle, *Laemophloeus ferrugineus* (Steph.), a serious pest of stored grain in Western Canada. Twenty-five adults, reared at 80° F. and 75% relative humidity (R.H.), were introduced into each jar of wheat. After 1 week under the same condi-

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[3] Malathion Grain Protectant, premium-grade. Chipman Chemicals Ltd., Winnipeg, Manitoba.

[4] Canadian Aviation Electronics Ltd., Winnipeg, Manitoba.

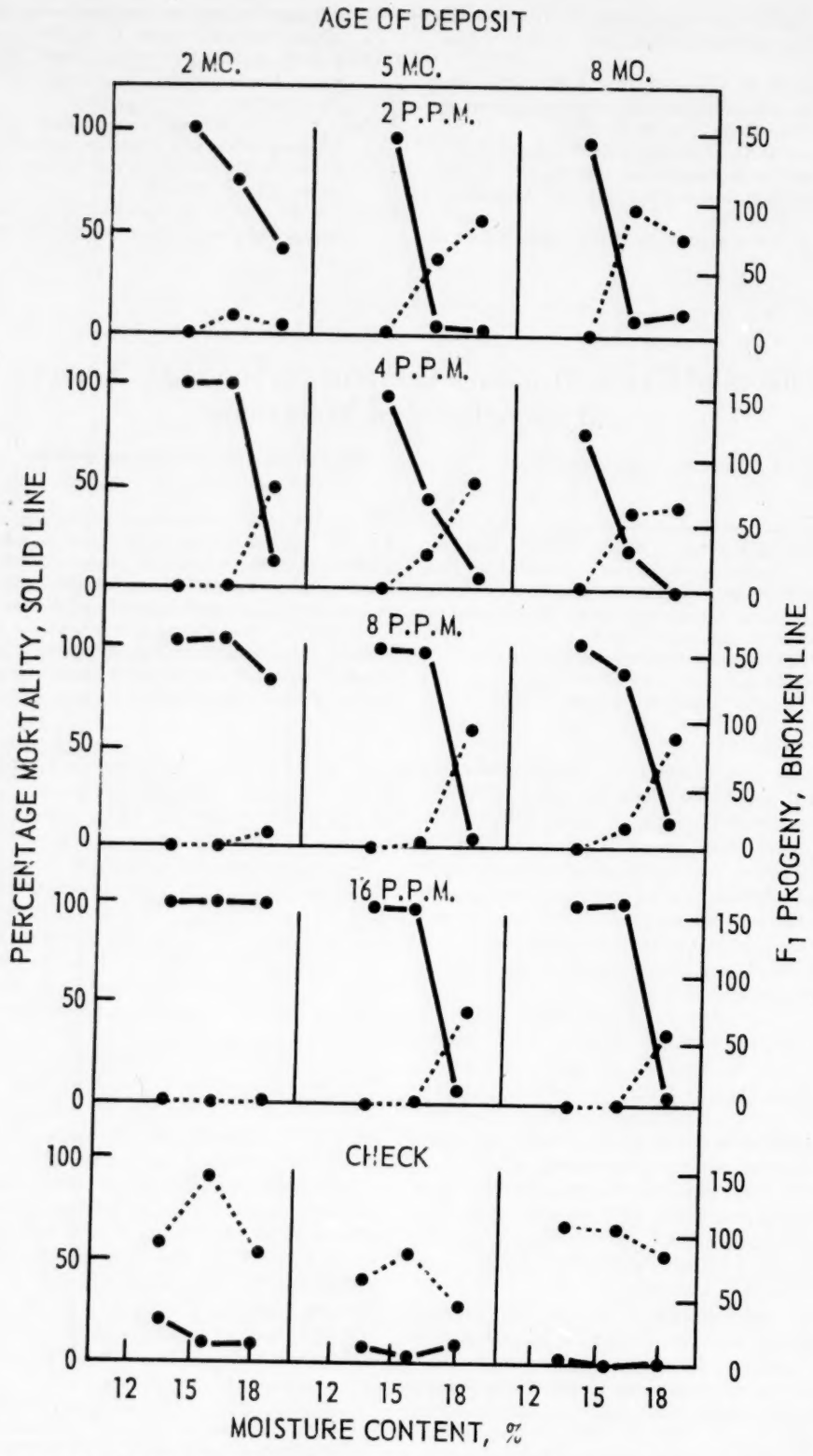

FIG. 1.—Percentage mortalities (solid lines) of adults of *Laemophloeus ferrugineus* (25 per sample) exposed for 1 week, in wheats of three different moisture contents, to various dosages of malathion each of three different ages, and numbers of F_1 progeny (broken lines) emerging. Difference necessary for significance at 1% level: mortality, 17.90%; progeny, 55.61.

tions, they were sifted and placed on fresh untreated wheat where they remained for 2 days at 80° F. and 75% R.H. before assessment of mortality. The jars were then stored at 80° F. for 50 days, after which the wheat was sifted to record emergence of F_1-generation beetles.

For tests on repellency, wheat was held in plastic boxes $2\frac{1}{2}\times8\times4$ inches, which were divided by a removable vertical slide into equal compartments $2\frac{1}{2}\times4\times4$ inches. The samples were conditioned with water to give 12, 15 and 17% moisture contents. Wheat treated at 4 or 8 p.p.m. of toxicant and aged 2 weeks was placed in one compartment; untreated wheat was placed in the other. Boxes with untreated wheat in both compartments were used as checks. Four replicates were used for each dosage at each moisture content. The test insects were *L. ferrugineus* and the granary weevil, *Sitophilus granarius* (L.). Twenty-five adults of each species were placed in each compartment of untreated wheat. The slide was then removed, allowing the insects freedom of movement between the treated and untreated portions. After 1 week, the vertical divider was replaced and the insects sifted from the wheat in each compartment. They were then placed on untreated wheat for 2 days, after which mortalities were recorded. The wheat was incubated at 80° F. and 75% R.H. for 50 days to obtain F_1-generation beetles.

Analysis of variance was applied to the data obtained for each species; the arcsine transformation was applied to the percentage mortality data.

Milling and baking tests were carried out on samples of untreated wheat and wheat treated with malathion at 4 and at 8 p.p.m. The test samples were neither cleaned nor scoured, and before milling were tested for odor. The samples were milled to approximately 72% extraction. Triplicate bakings were made by the malt-phosphate-bromate formula.

RESULTS AND DISCUSSION.—Figure 1 shows that wheat moisture content is an important factor influencing the effectiveness of malathion against *L. ferrugineus*. The adverse effect of high moisture content on malathion can be compensated for to some extent by an increase in dosage. However, there appears to be a limit since, although 8 p.p.m. gave mortalities of more than 86% at 8 months in wheat of 13.5 and 15.5%, doubling the dosage to 16 p.p.m. failed to give more than 5% mortality in wheat of 18% moisture content at 5 months after treatment. The numbers of F_1-generation beetles that emerged from the treated wheat were closely related to the mortalities of the test insects; but in some cases, even when adult mortality was high, there was some oviposition and normal development.

Malathion is a comparatively volatile insecticide, the vapor pressure as listed by the manufacturers (1955) being of the order of 4×10^{-5} mm. of Hg at 86° F. (99+%). It is possible, therefore, that storage of treated wheat in airtight containers at 48° F. prolonged effectiveness beyond the time expected had the samples been stored in open containers at room temperature.

In the tests on repellency, table 1 shows that at 12% moisture content, more *L. ferrugineus* were found in untreated wheat than in wheat treated at 4 p.p.m. This was the only indication that malathion repelled adults. When the moisture content was increased to 15%, the insects were evenly distributed; but at 17% moisture content, more were found in the treated wheat. At a dosage of 8 p.p.m., there was no significant difference between the numbers found in adjoining treated and untreated wheats at any moisture level.

There was no indication that *S. granarius* was repelled from wheat treated with malathion. The insects were evenly distributed in untreated wheat and wheat treated at 4 p.p.m., at 12 and at 15% moisture content. However, more were found in treated wheat at 17% moisture content. Also, at a dosage of 8 p.p.m., more adults were found in the treated wheat at all moisture levels. These results indicate that wheat treated with malathion may trap insects that move out of untreated portions. The insects were distributed evenly in both compartments of the untreated checks.

The generally high mortalities of both species in untreated and treated wheat (table 2) suggest that malathion may be highly effective against insects in wheat that has been unevenly treated. At 12% moisture content, more *S. granarius* were found dead in treated wheat at each dosage than in adjoining untreated wheat. Also, at all moisture levels, there were higher mortalities of *L. ferrugineus* in wheat treated at 4 p.p.m. than in the adjoining untreated wheat. At 8 p.p.m., at 17% moisture content, there was a higher mortality of *L. ferrugineus* in the treated wheat than in the untreated. Mortalities in the checks were low.

The habit of *S. granarius* of laying eggs within the

Table 1.—Numbers of living and dead adults of two species of grain insects 7 days after 100 of each species were placed in untreated wheat varying in moisture content and adjoining similar wheat treated with malathion.[a]

PER CENT MOISTURE CONTENT	*S. granarius*			*L. ferrugineus*		
	Treated		Untreated	Treated		Untreated
	Dosage: 4 p.p.m.					
12	59		32	28	<*	71
15	53		42	48		51
17	68	*>	33	74	**>	24
	Dosage: 8 p.p.m.					
12	78	**>	15	45		49
15	71	**>	19	36		54
17	71	**>	27	48		43

[a] Number in treated wheat significantly greater or less than that in untreated at the 1% (**) and 5% (*) levels, respectively.

Table 2.—Percentage mortalities of two species of grain insects 7 days after 100 of each species were placed in untreated wheat varying in moisture content and adjoining similar wheat treated with malathion.[a]

PER CENT MOISTURE CONTENT	*S. granarius*			*L. ferrugineus*		
	Treated	Untreated	Check[b]	Treated	Untreated	Check[b]
	Dosage: 4 p.p.m.					
12	93.8 *>	56.2	5.9	91.4**	>31.2	5.3
15	92.7	65.3	3.4	84.8**	>37.2	1.9
17	96.6	88.4	1.0	97.5 *>	72.9	0.9
	Dosage: 8 p.p.m.					
12	97.2 *>	67.2	6.0	100.0	84.9	3.1
15	100.0	89.6	5.2	94.4	82.1	5.5
17	100.0	91.9	0	100.0**	>67.7	2.2

[a] Mortality in treated wheat significantly greater or less than that in untreated at the 1% (**) and 5% (*) levels, respectively.
[b] Average mortality in four pairs of compartments.

Table 3.—Numbers of F_1-generation adults of two species of grain insects obtained from untreated wheat and wheat treated with malathion.[a]

Per Cent Moisture Content	*S. granarius*[b]			*L. ferrugineus*[b]		
	Treated	Untreated	Check[c]	Treated	Untreated	Check[c]
Dosage: 4 p.p.m.						
12	64 <*	108	167	0	0 <**	56
15	1 <**	357	466	0	3 <**	147
17	1 <**	340	640	1	0 <**	98
Dosage: 8 p.p.m.						
12	5	9 <**	57	1	1 <**	71
15	5 <**	90 <*	215	1	8 <**	140
17	2 <**	109 <**	344	1	10 <**	240

[a] The wheats were exposed in adjoining compartments to 100 adults of each species for 7 days before being incubated for 50 days at 80° F. and 75% relative humidity.

[b] Number in untreated wheat significantly greater or less than that in treated wheat or checks at the 1% (**) and 5% (*) levels, respectively.

[c] Average numbers in four pairs of compartments.

kernels affords protection for immature stages during development. Despite adult mortalities of 56.2 to 96.6% among the test insects after 1 week in wheat unevenly treated at 4 p.p.m. (table 2), reproduction (table 3) did not differ significantly from that in the checks, in which mortalities of 1 to 5.9% occurred. These results suggest accelerated oviposition among *S. granarius* exposed to low dosages of malathion in unevenly treated grain.

However, reproduction by *S. granarius* was reduced in wheat unevenly treated at 8 p.p.m.; significantly more F_1-generation adults were taken from the checks (table 3). Also, few adults were taken from each of the adjoining treated and untreated wheats at 12% moisture content; at the 15 and 17% levels, more adults were taken from untreated than treated wheat.

Although lower mortalities of *L. ferrugineus* were obtained in untreated wheat than in adjoining wheat treated at 4 p.p.m. (table 2), reproduction was low in both wheats (table 3); no significant differences between them were recorded. Eggs of *L. ferrugineus* are laid on the outside of wheat kernels or between them and the larvae were not as well protected from malathion during their development as those of *S. granarius*. Furthermore, *L. ferrugineus* lays fewer eggs than *S. granarius*. The results show that malathion applied unevenly to stored wheat at a dosage of 4 or 8 p.p.m. may be effective in limiting infestations of *L. ferrugineus*.

Malathion applied to wheat at dosages of 4 and 8 p.p.m had no adverse effect on quality (Anderson 1956). No foreign odor was detected during baking or in cut loaves.

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Time Between Puncture by the Boll Weevil and Fall of the Punctured Cotton Square[1]

R. E. Fye,[2] W. W. McMillian,[3] and A. R. Hopkins[2]

Abstract

Study of the time a cotton square remains on a plant after it has been punctured by a boll weevil (*Anthonomus grandis* Boh.) showed that there was no difference in this respect between Empire WR, Dixie King, and Coker 100 W varieties. The number of punctures and the type of puncture, whether feeding or oviposition, had little or no effect. Squares were retained an average of 11 to 13 days. Twenty per cent of the squares remained on the plant and produced usable lint. Plants treated with gibberellic acid retained more punctured squares than untreated plants.

The time that a square remains on the cotton plant after being punctured by a boll weevil (*Anthonomus grandis* Boh.) may be of concern in two opposing manners. If a punctured square is held on the plant and produces cotton lint of quality equal to that produced by unpunctured squares, a variety with this attribute may be suitable. On the other hand, if a punctured square is dropped readily and the plant quickly sets a new square in its place and thus re-establishes the original production potential for lint of equal quality, a variety with this tendency may be equally desirable. In areas where boll weevils are a major problem, the yield of varieties that do not exhibit either trait will undoubtedly be lower than that of varieties that do. To determine whether the varieties recommended and grown under conditions found in the coastal section of South Carolina possessed these characteristics, tests were undertaken in Florence, S. C., in 1956 and 1957.

Hunter & Hinds (1905) reported that a small percentage of punctured squares remained on the cotton plants and that most of the egg-punctured squares fell after an average period of 9.6 days. In South Carolina Fenton & Dunnam (1929) found the average time a square clung to the plant after a single egg puncture was 7.35 days. Following two egg punctures the square remained on the plant for 7.02 days, after three punctures 7.08 days, and after four to six punctures 6.53 days. No note of the time a square with feeding punctures remained on the plant was made by these authors, nor were the varieties in the studies mentioned.

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Table 1.—Time squares remained on cotton plants of different varieties after being punctured by the boll weevil.

Number of Punctures		Coker 100 W				Coker 100 W with Gibberellic Acid (1957)		Dixie King (1957)		Empire WR (1957)	
		1956		1957							
Feeding	Egg	Number of Records	Days	Number of Records	Days	Number of Records	Days	Number of Records	Days	Number of Records	Days
					Falling Squares						
1	—	256	11.0	281	13.4	72	11.6	279	12.9	262	12.7
2	—	26	10.9	22	13.2	2	16.5	15	12.5	23	11.8
3	—	10	13.2	—	—	—	—	1	8.0	5	10.6
4	—	3	5.0	—	—	—	—	1	14.0	1	21.0
	1	88	10.9	88	12.2	33	10.9	88	12.1	79	11.9
	2	1	4.0	—	—	1	10.0	2	11.5	1	5.0
	3	2	7.5	—	—	—	—	—	—	—	—
1	1	8	11.1	1	12.0	1	13.0	4	9.8	4	12.0
1	2	2	6.0	—	—	—	—	—	—	1	9.0
1	3	1	7.0	—	—	—	—	—	—	—	—
2	2	—	—	—	—	—	—	—	—	1	4.0
					Clinging Squares						
1	—	—	—	5	39.4	2	33.0	12	40.3	6	35.5
2	—	—	—	1	53.0	—	—	—	—	1	30.0
3	—	—	—	1	55.0	—	—	—	—	—	—
4	—	—	—	—	—	—	—	—	—	1	31.0
—	1	—	—	2	34.5	1	34.0	6	37.8	1	40.0

METHODS.—In the tests at Florence squares on 25 plants of each recommended variety were examined daily during the normal fruiting period. When a square was punctured, it was tagged with a number and the date and number and type of puncture were recorded. Daily examinations for dropped squares were made, and the date of drop and other pertinent information were recorded.

Coker 100 W was observed in 1956, and Coker 100 W with and without weekly sprayings of 1:10,000 gibberellic acid, Dixie King, and Empire WR varieties in 1957.

RESULTS AND DISCUSSION.—The number of records of falling squares and the number of days punctured squares remained on the plant are presented in table 1.

The records for Coker 100 W show little difference between the two years in the average time between puncture and drop. Since climatic conditions play a considerable role in determining this period, the small differences between the 2 years are attributable to these factors. In 1956 violent thunderstorms were common, and drop during and after these storms was heavy. In addition, the excessive moisture of these storms followed a drought period and apparently physiological shed was high after the storms occurred. This square abscission caused by the rapid change in moisture conditions may have accelerated the drop due to weevil punctures. In 1957 several storms occurred, but they were not as violent and did not follow a drought period.

Squares that remained on the plants more than 28 days and then dropped as small developing bolls without further puncture were considered as clinging. The presence of clinging squares in 1957 and not in 1956 may further indicate that under more ideal climatic conditions punctured squares may be retained for longer periods. No emergence of adults from clinging squares or the small developing bolls was observed.

Coker 100 W, Dixie King, and Empire WR varieties all retained punctured squares for about the same period. The small differences indicated in the table are attributable to the limitations of the experimental methods.

There is apparently very little difference in time elapse for egg and feeding punctures.

The period between puncture and drop also appears to be unaffected by the number of punctures in the square. The exception to this is a shortened period when, due to high population pressures, several feeding punctures are placed in squares that are so small that they ordinarily would be passed over by feeding weevils or ovipositing females.

Table 2 indicates that the three varieties drop approximately the same percentage of punctured squares and retain the same percentage of clinging squares. Most noteworthy is the fact that from 19 to 32% of the punctured squares were retained by the plants and produced bolls. The records showed that most of these retained squares had been punctured for feeding purposes. Apparently the feeding was superficial and had not reached plant ovaries or other tissue involved with the retention of the square. Squares with up to 10 feeding punctures were retained and commonly produced bolls with some usable lint. From 4 to 7% of the punctured squares produced normal

Table 2.—History of boll weevil-punctured squares in cotton.

Variety	Per Cent		Per Cent Maturing Bolls				
	Falling	Clinging	Normal	Partially Destroyed	Wholly Destroyed	Condition Influenced by Later Puncture	Total
Coker 100 W:							
1956	80.7	0	5.6	6.6	3.2	3.9	19.3
1957	74.1	3.4	4.3	2.6	2.9	12.7	22.5
1957[a]	66.5	1.8	5.5	3.7	5.5	17.0	31.7
Dixie King (1957)	72.2	3.3	5.7	5.3	3.5	10.0	24.5
Empire (1957)	72.6	1.8	7.4	3.1	2.9	12.4	25.6

[a] With gibberellic acid.

bolls, and from 2 to 7% produced bolls that were partially destroyed. Only 3 to 5% of the squares that matured into bolls were totally destroyed and had no usable lint. The final condition of a high percentage of the retained squares that matured were influenced by later punctures. Egg punctures were most commonly superimposed on the earlier feeding punctures and at maturity damage by the larvae was noted. Usable lint was taken from all the bolls thus affected.

Gibberellic acid had some effect upon the fall of squares, for the retention by the treated plants was greater than in the untreated. However, the differences may have been within the experimental error. The greatest effect of the gibberellic acid was in increasing the number of punctured squares that matured to bolls. This difference is probably greater than can be attributed to experimental technique, but more critical experiments must be conducted before its complete effect can be ascertained.

It is apparent from these studies that there is little difference in the cotton varieties recommended in the State of South Carolina and produced under coastal plain conditions in respect to their retention of punctured squares. None of the three show superiority in retention to maturity or rapid drop with immediate replacement of punctured squares. There is some indication that chemical treatment may improve punctured square retention.

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The Colonization and Establishment of Imported Parasites of the Spotted Alfalfa Aphid in California[1]

R. VAN DEN BOSCH, E. I. SCHLINGER, E. J. DIETRICK,[2] K. S. HAGEN,[3] and J. K. HOLLOWAY[4]

ABSTRACT

During 1955 and 1956 three parasites of the spotted alfalfa aphid, *Therioaphis maculata* (Buckton), were obtained from Europe and the Middle East. These parasites, *Praon palitans* Muesebeck, *Trioxys utilis* Muesebeck (both Braconidae), and *Aphelinus semiflavus* Howard (Eulophidae), were successfully mass produced in the insectaries at Albany and Riverside, California, and released in large numbers at many localities throughout the state. Initial efforts to effect parasite establishment, in commercial alfalfa were greatly hampered by insecticidal treatment of the fields, interference from native coccinellids and fungus disease of the aphid, and the disruptive effects of the alfalfa harvesting process. However, heavy build-up of parasites in a controlled plot near Lancaster, California, in the summer of 1956, and the subsequent distribution of the parasites from this plot on cut alfalfa, resulted in widespread parasite establishment in southern California by the end of the year. In 1957 parasites were colonized in all *T. maculata*-affected areas of California, mainly with material collected by a large truck-mounted mechanical collector. By the end of 1957 parasites were established in areas involving about 1,000,000 acres of irrigated alfalfa and were playing an increasingly important role in the biological control of *T. maculata*.

Within a year of the first damaging outbreaks of the spotted alfalfa aphid, *Therioaphis maculata* (Buckton), in California, it had been determined that this new pest was an accidental immigrant from the Old World (Dickson *et al.* 1955), and that it was commonly attacked by hymenopterous parasites in its native home (letter from I. Harpaz, Hebrew University, Rehovot, Israel, to R. C. Dickson, University of California, Riverside, dated April 8, 1955) (Harpaz 1955). On the basis of this information a search for parasites was initiated in Europe, Africa, and Asia in the late spring of 1955 (Clausen 1955). As a result of this effort, which was a joint undertaking of the Agricultural Research Service, United States Department of Agriculture and the Department of Biological Control, University of California, three hymenopterous parasites were obtained from *Therioaphis* spp. and eventually placed in mass production in the insectaries of the Department of Biological Control, University of California, at Riverside and Albany (van den Bosch 1956, 1957). These parsites, *Praon palitans* Muesebeck, *Trioxys utilis* Muesebeck (both Braconidae) (Muesebeck 1956), and *Aphelinus semiflavus* Howard (Eulophidae), were subsequently produced in very large numbers and colonized at many localities throughout the major alfalfa-growing areas of the state. Ultimately all three species became widely established in the field (van den Bosch *et al.* 1957, Hagen *et al.* 1958).

Aside from the establishment of *Aphelinus mali* Hald. on the woolly apple aphid, *Eriosoma lanigerum* (Hausm.), in several countries (Clausen 1956), and *Aphidius* (*Lysiphlebus*) *testaceipes* (Cresson) on a number of aphid species in Hawaii (Williams 1931), to the knowledge of the authors no other successful importation and establishment of an aphid parasite has been recorded in the literature. For this reason the establishment of the three imported parasites of the spotted alfalfa aphid in California during 1956 is felt to be of considerable interest and importance.

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PARASITE COLONIZATION METHODS.—In its invasion of California, *Therioaphis maculata* first appeared in the valleys of the Colorado Desert in the southeastern corner of the state in 1954. It moved rapidly over southern California during that year, and in 1955 it was generally severe over all the area south of the Tehachapi Mountains (Dickson *et al.* 1955, Smith *et al.* 1956). By late spring of 1955 it had also moved into the Central Valley of California, and by the fall of that year it had attained severe economic status in several important alfalfa-growing counties of central California. The next year *T. maculata* became well distributed over all the major alfalfa-producing areas of California and its damage was generally severe. Parasite colonization was initiated in southern California in the late summer of 1955 with material produced in the insectary at Riverside. Several colonizations of Riverside-reared material were also made in central California, but inasmuch as insectary propagation of parasites could not be undertaken at Albany at that time (because of quarantine restrictions in Alameda County) colonization in this area was very limited.

In the initial stages of the parasite colonization program relatively small numbers of parasites were released rather indiscriminately in commercial alfalfa fields in both southern and northern California. Despite numerous releases of this type on heavy aphid infestations in the summer and fall of 1955, no parasites were recovered after several months' intensive search, and a change in colonization method was deemed necessary. In effecting this change, the random distribution of parasites was abandoned and a number of "permanent" colonization plots were established. Furthermore, with increased insectary production the sizes of releases were considerably enlarged, and it became possible to colonize the same plots repeatedly. Following these releases, recoveries of all three parasite species were made in southern California in the spring of 1956, and *Aphelinus semiflavus* was recovered in northern California later that year. These encouraging developments indicated that parasite establishment might soon be effected. However, a series of catastrophes befell the majority of these release plots, and eventually essentially all colonies were lost. Exceptions appear to have been the successful establishment of *Trioxys utilis* near Winterhaven on the California-Arizona border in eastern Imperial County, and *Praon palitans* in Tehama County in the northern Sacramento Valley. Two of the most important factors which contributed to the widespread loss of parasite colonies in the spring of 1956 were insecticidal treatment of some of the release plots and the surrounding acreages, and the depletion of aphid infestations in the plots by native coccinellids or fungus disease. Other contributing factors beyond the control of the authors were the mowing or grazing off of the plots, and in several cases the plowing under of the alfalfa.

Thus, in southern California despite the fact that parasites had gained considerable momentum in 19 out of 47 release plots in the spring of 1956, they had not become abundant enough or well-distributed enough in the great majority of these plots to survive the severe effects of the disturbances mentioned above.

These experiences indicated a need for colonization plots over which absolute control could be exercised so that conditions as near optimum as possible for parasite survival and reproduction could be maintained. Fortunately, a plot which met these requirements was obtained on the farm of R. L. Reynolds, 8 miles east of Lancaster in the Antelope Valley, an alfalfa-growing region of about 40,000 acres on the southwestern edge of the Mojave Desert. This grower gave assurances that he would not, under any circumstance, apply insecticides to any portion of his 38 acres of alfalfa. He further agreed to set aside an area of approximately 5 acres as an experimental area in which any type of manipulation or cultural practice deemed necessary to favor establishment of the parasites could be employed. Within the 5-acre experimental area approximately 1 acre was established as the parasite release plot and was left uncut during the entire colonization program. The remaining portion of the 5-acre area was to be used as a parasite "nursery" if and when spread occurred from the 1-acre release point. This was to be accomplished through staggered cutting of the alfalfa which, it was felt, would favor the continuous occurrence of aphids in the area.

Perhaps the key factor leading to the ultimate establishment of parasites in the Reynolds plot was the placing of a large organdie cage (6′×6′×3′ in dimension) in the center of the plot into which the parasites were released. This was done in order to eliminate coccinellid predation on the aphid population. To further favor the parasites, stocks of aphids were periodically introduced into the cage to maintain a constantly high infestation level within the confined area. During the period of colonization, April through June 1956, releases were made at about weekly intervals with 14,400 *Praon palitans*, 5,860 *Trioxys utilis*, and 6,250 *Aphelinus semiflavus* being introduced into the cage. Similar releases were also made during the same period into a cage in a plot on the W. B. Camp ranch at Cawelo, Kern County, in the southern San Joaquin Valley.

At Lancaster by mid-June aphid mummies produced by all three parasite species were abundant within the confined area and the cage was then opened to permit the spread of the adult parasites from this focus. Simultaneously, the alfalfa in the surrounding area was placed on a staggered cutting cycle to favor continuous availability of aphids to the parasites. Fortunately, initial parasite build-up occurred at a time when the aphid population was on the upsurge in the 38-acre alfalfa field and coccinellid activity was at a minimum. Under these conditions the parasites spread very rapidly, quickly established themselves throughout the 5-acre experimental area, and then moved into the commercial alfalfa adjoining this area.

At Cawelo parasites also built up within the cage, but the experimental area consisted of only about one-quarter acre of alfalfa which was surrounded on all sides by heavily treated commercial acreages. As a result, the parasites flying into treated alfalfa in which aphid populations were periodically depleted never gained the momentum that they did at Lancaster.

By mid-July at Lancaster all three parasite species had gained enough reproductive momentum, and had spread sufficiently, to survive an aphid scarcity caused by a belated coccinellid attack and to pass through the

cutting cycle in the commercial portion of the field. By the first of September both *Praon palitans* and *Trioxys utilis* had become extremely abundant throughout the 38 acres of alfalfa on the Reynolds farm and had spread to fields adjoining this acreage.

At this time a decision was made to attempt parasite establishment in several new areas by moving freshly cut alfalfa from the Reynolds plot. It was reasoned that since cocooned parasites (in mummified aphids) were common on the plants (averaging 40 per stem in some places), and since additional millions of parasitized live aphids occurred on the alfalfa, such transfers afforded an unprecedented opportunity to make massive parasite introductions into other major alfalfa-growing areas. Such colonizations, if effective, would, of course, greatly hasten the spread of parasites over the *T. maculata*-affected areas of California.

This program was made possible through the cooperation of the several county agricultural commissioners who sanctioned the movement of the aphid-infested alfalfa under controlled conditions into their counties, and a number of growers who permitted establishment of plots in their fields. Colonizations were made at key localities in Kern, Los Angeles, San Bernardino, Riverside, San Diego, and Imperial counties in southern California. An estimated 50 million cocooned parasites and countless additional millions within parasitized live aphids were transferred to these new areas on the cut alfalfa obtained from the Reynolds farm (van den Bosh *et al.* 1957). Invariably, where these heavy colonizations were made, establishment of the parasites was effected. Subsequent movements of parasite-bearing alfalfa from the new establishments to additional plots in late 1956 resulted in even further spread of the parasites in the field by the end of the year.

Since the cutting, loading, and transporting of alfalfa was a laborious and costly process, it was decided at the end of 1956 that a more efficient method should be developed for the 1957 colonization activities. Accordingly, during the winter of 1956–57 one of the authors (E. J. Dietrick) conceived, perfected, and made ready for use a mechanical parasite collector. This mechanical collector (fig. 1), which was mounted on a half-ton pick-up truck, consisted of a 15-foot metal scoop placed at the front of the truck, and two aluminum-ribbed canvas air ducts running from the scoop to a large rotary fan located in the truck bed. Contained within each air duct were two insect-collecting sacks, an inner one of coarse mesh nylon and an outer one of organdie. The fan was powered by a gasoline motor. When the collector was driven through an alfalfa field, insects were knocked onto the scoop and then drawn into the collecting sacks in the air ducts by an air stream generated by the fan. The coarse mesh nylon sacks screened off the larger arthropods and debris, while the parasites, parasitized aphids and other small insects and debris passed through to the outer organdie sacks. At intervals the collecting sacks were removed and the contents packaged and held under refrigeration until releases were to be made. The collector, on occasion, was driven over 30 to 40 acres of alfalfa in a day and rapidly collected millions of parasites when conditions were favorable. Colonizations of material obtained by the mechanical collector were made in the following two ways: (1) as local transfers in which packaged parasite material was distributed on field days to growers in localized farming areas for release by themselves, and (2) as area-to-area transfers in which massive releases of parasites were made in colonization plots geographically remote from the source areas. It is estimated that during 1957 somewhat in excess of 100 million parasites were transferred by these two methods. More than 350 growers in four major alfalfa-growing areas received material at field days, while about 100 widely distributed plots were colonized in the area-to-area transfers. These transfers, though they probably did not invariably result in establishment of the parasites, were nevertheless highly successful, for with so many colonizations being involved the chance for complete failure of establishment in any given area was practically nonexistent. Colonizations made with material collected by the mechanical sweeper effected establishment of parasites in the Borrego Valley, Palo Verde Valley, Owens Valley, Kern River Canyon, Salinas Valley, northern San Joaquin Valley, and the Sacramento Valley during 1957. It is felt that the combined colonization effort involving parasite-bearing alfalfa cuttings and mechanical-sweeper-collected material hastened the spread of the *T. maculata* parasites over California by many months if not several years.

KNOWN RANGE OF PARASITES AT THE END of 1957.—The massive colonization efforts undertaken in late 1956 and during 1957 effected establishment of imported splotted alfalfa aphid parasites in every major alfalfa-growing area of California (van den Bosch *et al.* 1957, Hagen *et al.* 1958). Thus, in a little more than a year from their initial build-up at a single locality in the Antelope Valley, the parasites had become established at numerous localities in areas involving about 1 million acres of irrigated alfalfa. These localities ranged from Imperial County on the southern border of the state to Tehama County in the northern Sacramento Valley, and from coastal San Diego County in the west to the valleys along the Colorado River in the east. Conservatively, this is estimated to comprise a geographical area of about 100,000 square miles. By the end of 1957 the parasites had spread naturally from localized foci over considerable distances in several areas, and parasitism had become an important factor in the biological control of *T. maculata* at some places. However, the range of each parasite species differed from that of the others (fig. 2) and these will therefore be discussed below.

Praon palitans.—This parasite made the greatest progress of the three, in so far as spread is concerned, apparently because it was carried in proportionately greater numbers by parasitized alate aphids than either *Trioxys utilis* or *Aphelinus semiflavus* (van den Bosch *et al.* 1957, Schlinger & Hall 1959). The rapidity of spread of *Praon palitans* was rather strikingly demonstrated in the Mojave Desert in the fall of 1956 when this species was carried by parasitized winged aphids from the Antelope Valley to the Mojave River Valley, a distance of about 40 miles. Similar distances were covered in the southern San Joaquin Valley where *P. palitans* spread from a single colonization site over an area of about 700 square miles in the fall of 1956.

By the end of 1957 *Praon palitans* was established over the entire southern portion of the Central Valley of

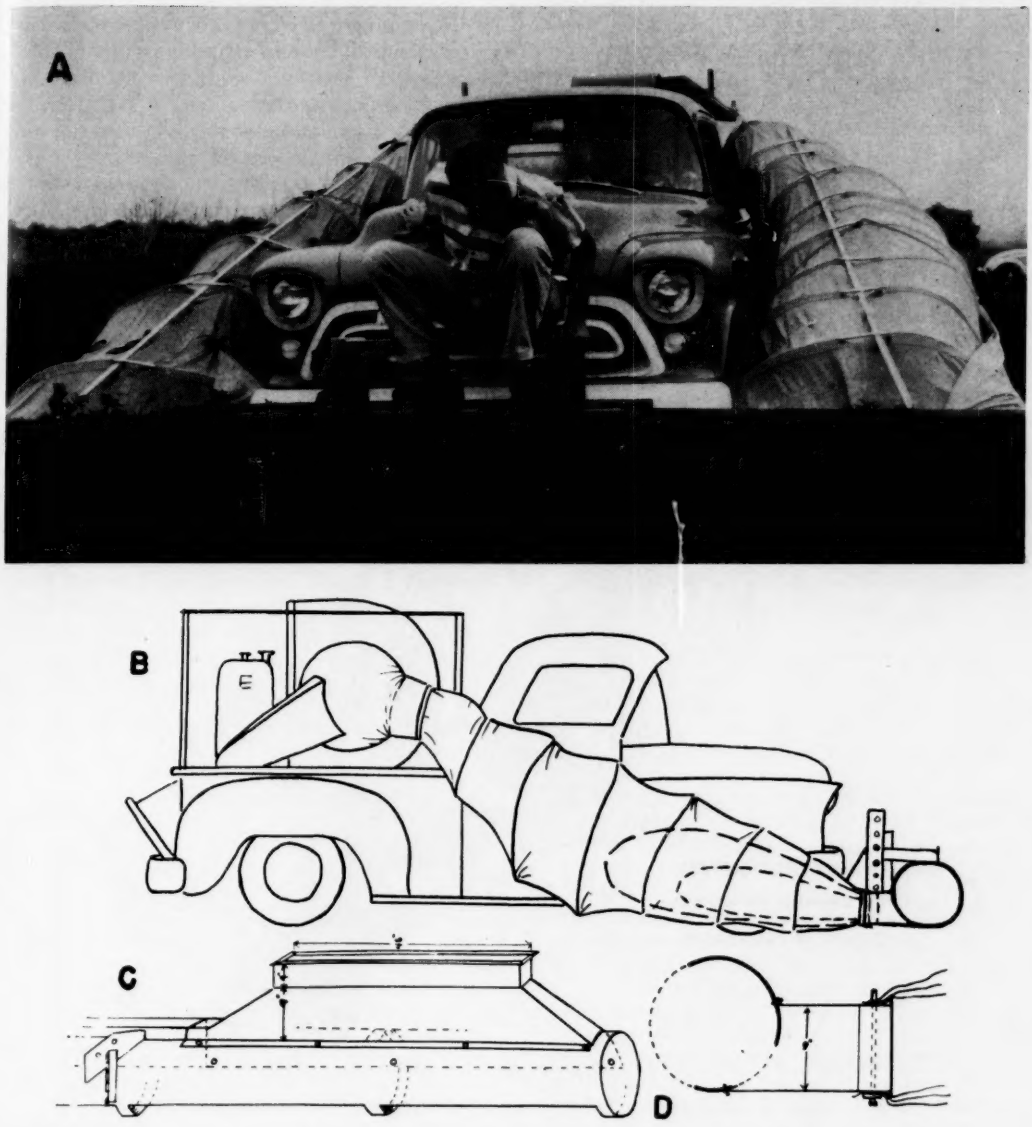

FIG. 1.—Mechanical collector developed for large-scale field collection of imported parasites of the spotted alfalfa aphid: (A) collector in operation; (B) diagrammatic side view of collector—broken line invaginations in air duct indicate positions of collecting sacks; (C) diagram of one side of "scoop" showing the adaptive collar with which it is connected to air duct; (D) lateral view of "scoop"—note anterior opening (broken line) for entry of insect material and posterior opening through which material is drawn via adaptive collar into collecting sacks in air duct.

California, and at scattered points from Stanislaus County north. It also occurred in all the alfalfa-growing areas of the Mojave Desert, as well as in the Owens Valley, the southern part of the Salinas Valley, and over much of the south Coastal Plain. However, its status in the alfalfa-growing valleys of the Colorado Desert remains obscure, with sporadic recoveries indicating that it may be widely distributed in this area but extremely scarce. There are increasing indications that this parasite is poorly adapted to areas with intensely hot summer climates, as appeared to be the case in the Middle East (van den Bosch 1957).

Trioxys utilis.—By the end of 1957 this parasite had become particularly abundant and widespread in the valleys of the Colorado Desert, certain areas of the south Coastal Plain, and in the southern portion of the San Joaquin Valley. Localized foci also occurred in the Mojave Desert areas, the Owens Valley, and in Yolo and Stanislaus counties in central California. It quite probably occurred at scattered localities in other alfalfa-growing areas of the state, but had not reached great enough abundance to be detected in the periodic surveys.

Aphelinus semiflavus.—This species has been known from California for many years, having been recovered from time to time from several aphid hosts (Muesebeck *et al.* 1951). However, prior to the colonization of the material derived from Old World stocks, this parasite was encountered on only a single occasion on the spotted

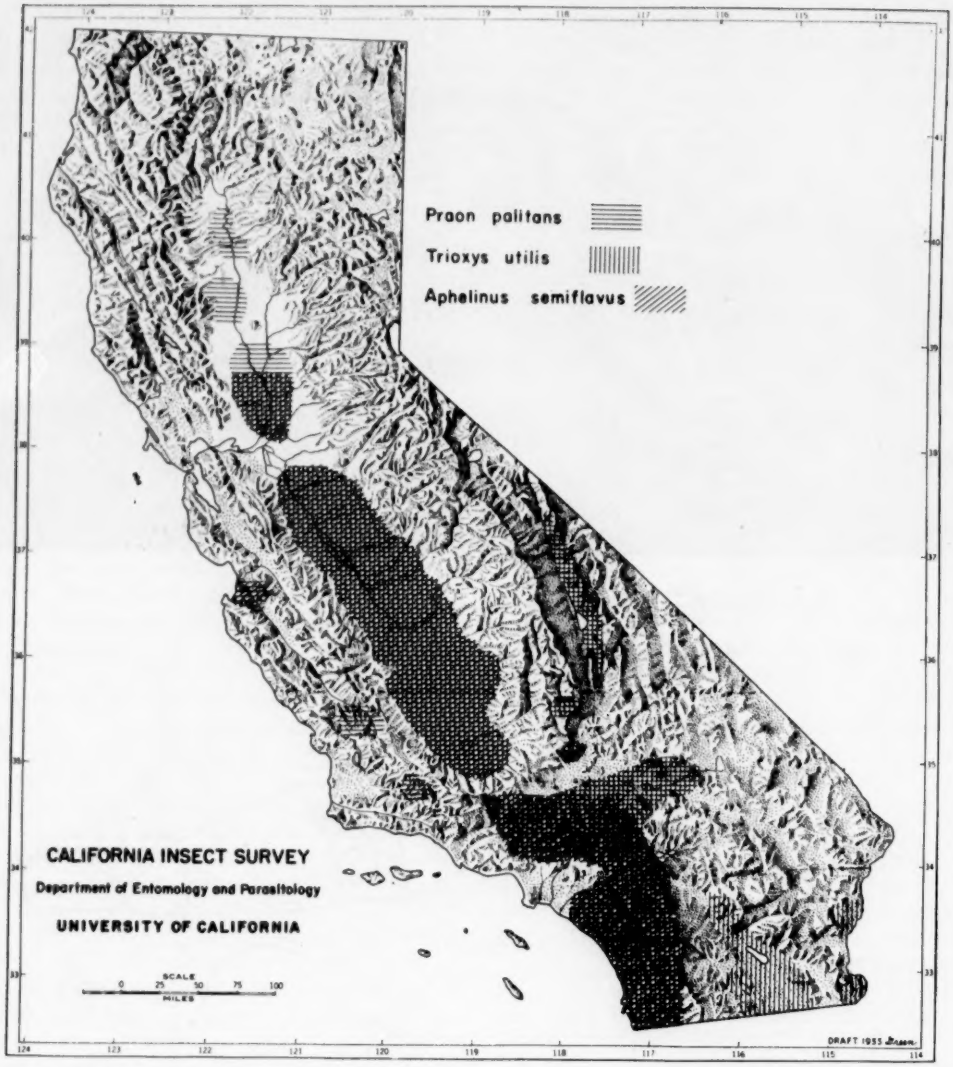

FIG. 2.—Known range of imported parasites of the spotted alfalfa aphid in California at the end of 1957.

alfalfa aphid in the field (unpublished record by T. W. Fisher of the Department of Biological Control, University of California, Riverside). Following the release of the insectary-reared material, recoveries of *A. semiflavus* were made with increasing frequency. In the vicinity of a number of release plots it continued to occur in considerable abundance through the end of 1957. Although the matter has not been adequately investigated, it is felt that the field performance of the imported *A. semiflavus* in California indicates it to be a distinct biological strain from the native form (Schlinger & Hall 1959). Generally, *A. semiflavus* appeared to be the least abundant and least important of the imported spotted alfalfa aphid parasites at the end of 1957. Nevertheless, it was known to be established at widely scattered locations in most of the major alfalfa-growing areas of California. It was encountered in greatest abundance at several localities on the south Coastal Plain, and on occasion was also found to be fairly common in the Central Valley. It was extremely scarce in the Colorado Desert Valleys and all evidence indicates that it will not attain important status there.

DISCUSSION.—Perhaps the most significant feature of the spotted alfalfa aphid parasite colonization program was the failure to effect widespread establishment during the first year of the program, and the ultimate solution of this problem following the adoption of the controlled release plot at Lancaster. Needless to say, the repeated failures which characterized the early colonization efforts were highly puzzling and discouraging to those connected with the program, for on a number of occasions parasites were released under what seemed to be very favorable conditions. However, when, in retrospect, one considers the catastrophic factors which worked against the parasites, *i.e.*, frequent decimation of aphid populations by coccinellids, disease, insecticides, adverse climatic conditions and the alfalfa harvesting activity, etc., it is no wonder that the widely scattered handfuls of parasites rarely gained the reproductive momentum to survive these hardship periods. Indeed it was only in a single plot, the one at Lancaster, in which every conceivable measure

was taken to favor the parasites, that rapid, heavy localized build-up occurred. Furthermore, it is questionable even in this case whether these favorable results would have been attained if the fortuitous circumstance of aphid build-up during a period of minimal coccinellid attack had not occurred in the area outside the colonization cage. It was also extremely fortunate that the grower refused to apply insecticides to any portion of his acreage, for it was on the heavy *T. maculata* infestation that developed over the entire 38 acres that the parasites gained the reproductive momentum that enabled them to spread into the surrounding alfalfa fields.

The success in effecting widespread parasite establishment with the alfalfa cuttings obtained at Lancaster in the fall of 1956 and alfalfa sweepings in 1957 gives strong support to the feeling that in this project, as well as in many others involving imported entomophagous insects, a minimum "inoculation charge" is needed if reasonable chance for parasite establishment is to be expected. Thus, in the cases involving the Lancaster alfalfa cuttings, where each colonization consisted of millions of parasites of all growth stages, these massive "charges" were sufficiently large to insure parasite establishment in every field where they were made, despite the fact that in a number of plots aphid populations were extremely light at the time of release or soon thereafter.

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The Role of Imported Parasites in the Biological Control of the Spotted Alfalfa Aphid in Southern California in 1957[1]

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Abstract

The spotted alfalfa aphid, *Therioaphis maculata* (Buckton), is attacked by three imported parasites in southern California. These parasites, *Praon palitans* Muesebeck, *Trioxys utilis* Muesebeck, and *Aphelinus semiflavus* Howard, were studied in three alfalfa fields in three distinct climatic areas in 1957. The roles of native coccinellids and fungus diseases (principally *Entomophthora exitialis* Hall & Dunn) in the biological control of the aphid were also evaluated. *T. maculata* was under heavy biotic pressure in all three fields over most of the year. In two of the fields, located at Cawelo and Lancaster, California, the aphid reached economic abundance on only one occasion. In the third field, located at Calexico, California, it did not reach economic status at any time during the year. Coccinellids appeared to play the most important role in the biological control of the aphid in all three fields. Disease was of major importance at Calexico. Parasitization reached high levels in each of the plots at certain times and contributed significantly to the biological control of the aphid at each locality. The alfalfa harvesting process appeared to have an adverse effect on the parasites, particularly in midsummer. Periodic invasion of the study fields by large numbers of alate *T. maculata* had an unfavorable effect on the parasite-host ratio. The parasites seemed to show varying climatic adaptation. *P. palitans* was dominant at Cawelo and Lancaster, while *T. utilis* was dominant at Calexico. *A. semiflavus* was encountered in fair abundance at Cawelo only.

During 1955 and 1956 a very intensive colonization campaign was carried on in California to attempt establishment of three imported parasites of the spotted alfalfa aphid, *Therioaphis maculata* (Buckton), (van den Bosch 1956). This campaign was ultimately successful, and all three species, *Praon palitans* Muesebeck, *Trioxys utilis* Muesebeck, and *Aphelinus semiflavus* Howard, became widely established in the state (van den Bosch *et al.* 1957, Hagen *et al.* 1958, van den Bosch *et al.* 1959).

Establishment was first effected in southern California, and by the end of 1956 the parasites had reached great enough abundance in several localities in this part of the state to show considerable promise in the biological control of the aphid (van den Bosch *et al.* 1959). Because of this condition a limited parasite evaluation program was carried out during 1957. The data gathered in these preliminary studies form the basis for the current paper. Much more thorough investigations of the biological control of *T. maculata* are now under way, and these will be discussed in a comprehensive publication at a future date.

Therioaphis maculata reached its peak destructiveness in southern California in 1955, when its estimated cost to alfalfa growers in seven counties south of the Tehachapi Mountains was approximately 5.75 million dollars. In 1956 the estimated aphid cost for the same counties declined to about 2.75 million dollars, and in 1957 it dropped even further to about 1.25 million dollars.[2] The diminished severity of the *T. maculata* problem in southern California can be attributed, in part, to increased efficiency in chemical control methods. However, there is abundant evidence that biotic mortality factors contributed just as significantly, if not more so, to the improved situation. Among the biotic factors affecting *T. maculata*, native coccinellids and fungus disease, principally *Entomophthora exitialis* Hall & Dunn (1957), appear to have played the major roles. The imported parasites have thus far played secondary roles in the biological control of *T. maculata*, but they are rapidly increasing in importance. The current paper, though primarily concerned with the imported parasites, also discusses the roles of coccinellids and disease in the study plots, since this is important to an understanding of the efficacy of the parasites.

Methods.—The evaluation plots were established in three climatically distinct alfalfa-growing areas—the Imperial Valley (Calexico), the Antelope Valley (near Lancaster), and the southern San Joaquin Valley (Cawelo). Surveys were made at approximately weekly intervals during the warmer portions of the year, *i.e.*, late spring, summer, and early fall, and at approximately fortnightly intervals during the period of cooler weather. On each sampling date a stem count of living and parasite-mummified aphids was made and sweeps were taken. Fungus disease of the aphid was also rated. Originally five scattered samples of 20 stems each were examined in each field, making a total of 100 stems per field. Sweepings were also taken at these five scattered points in 20 sweep subsamples, making a total of 100 sweeps for each field. Later in the year it was found desirable to alter the sampling technique to the extent that the examination of a predetermined number of stems (100 per field) was abandoned and a time-sampling system substituted. Under the revised system stem counts were made by two samplers at four subsampling stations in each field. The time spent at each sampling station varied according to the aphid and parasite population densities. Consequently, on different sampling dates the total time spent in a field varied from one-half hour to 2 hours, depending on the population densities. Net sweeps were taken in 25 sweep increments at each of the four sampling points, making a total of 100 sweeps per field.

The time-count method of stem sampling was advantageous because it was adjustable to variations in aphid population density, thus enabling the samplers to examine greater or lesser numbers of stems, depending on the

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[2] These data were obtained from special reports of the Bureau of Entomology Department of Agriculture, State of California.

aphid infestation levels. With the predetermined stem count it was found that at high aphid densities sampler fatigue developed. Furthermore, superfluous data were often obtained at the high densities. At low densities, on the other hand, data were often insufficient. Under either method alfalfa stems were cut at ground level with a sharp knife, and all living and parasite-mummified aphids occurring on the stems were then counted. Living aphids were placed in four categories: alate adults, apterous adults, large nymphs, and small nymphs. Parasites, which occurred on the stems in the cocoon stage (*i.e.*, within mummified aphids), were recorded as to species, and it was also noted whether the mummies were formed from winged or apterous aphids, whether the parasites had emerged or not, and in the case of *Praon palitans* whether the mummies were diapause type or not. Parasitization was calculated on the basis of mummified aphids versus live adult aphids in the stem sample. This method was adopted in preference to the rearing or dissecting of aphids because of the erratic survival of reared aphids in the laboratory and the tediousness of dissection.

Two major disadvantages were inherent in the mummy comparison method of estimating parasitization. The first of these was a lag factor representing the developmental period between time of parasite oviposition and time of cocooning (*i.e.*, mummification of the aphid). Thus, in the graphed data parasitization recorded for any particular date developed from oviposition which may have occurred anywhere from several days to several weeks prior to the date that the aphid mummies were observed, depending upon temperatures. The second major disadvantage relates to the probability that during windy or rainy periods *Trioxys utilis* mummies, in particular, were probably dislodged from the plants in considerable numbers. Under such conditions the estimate of parasitization was conservative. Another factor, which unquestionably caused loss of mummies from the stem counts, was the dropping from the plants of the older infested leaves bearing mummified aphids. Finally, those aphids parasitized as adults were probably missed in the sampling particularly late in the cuttings since there frequently was not time for them to become mummified before harvest. Despite these drawbacks, it was felt that the advantages of speed and simplicity made the mummy comparison method the most desirable technique for estimation of parasitization in the field.

Disease was estimated by a rating system which was adopted because of the great difficulty in counting accurately the numbers of diseased *T. maculata* on the stems. Under the rating system five categories were established, these being: category 0, no disease; category 1, 1 to 25% disease; category 2, 26 to 50% disease; category 3, 51 to 75% disease; and category 4, 76 to 100% disease. Disease incidence was rated on each stem, and by accumulating these numbers and dividing the total by the number of stems examined, a figure ranging from 0 to 4 was obtained on each date. An estimate of disease percentage was then obtained by dividing this figure by 4. It is recognized that this method of calculating disease percentages had certain shortcomings, but it is felt to have given a reasonably accurate measure of disease incidence in each of the plots over the year.

Sweepings were taken to measure the abundance of coccinellids, parasite adults, a variety of predators, and various other insects associated with *T. maculata*. The population trends of syrphids and such general predators as *Nabis* spp., *Geocoris* spp., *Chrysopa* spp., etc., are not presented in the accompanying graphs because evidence could not be obtained that they played significant roles in the control of *T. maculata* in the study plots. Originally the swept insects were killed by cyanide and then placed in ice-cream cartons and counted in the laboratory. Later in the year, wide-mouth mason jars containing alcohol (approximately 40%) were used for killing and transporting the swept insects. The alcohol-collecting method was an improvement over the cyanide method inasmuch as the collected material could be held indefinitely in excellent condition even when great amounts of insect material were swept.

POPULATION TRENDS.—*Calexico, California* (Figs. 1 and 2).—Calexico is located in the Imperial Valley of southern California at the Mexican border. Climatically the Imperial Valley is characterized by intensely hot summers, mild winters and low annual rainfall. Long-term United States Weather Bureau data (U.S.D.A. Yearbook 1941) list the average temperature at Calexico for July as 89.8° F. and for January as 53.2°F. Frosts occur on occasion between December 9 and February 1. Annual rainfall averages slightly over 3 inches with the greatest amount falling from November through March. However, about 20% of the yearly precipitation occurs during thunder showers in late summer.

Africa is the alfalfa variety universally grown in the Imperial Valley with about 160,000 acres being planted there. Alfalfa does not become dormant during the winter in this area, and *T. maculata* is active upon it throughout the year.

The studies at Calexico were actually made in two fields with data obtained up to July being taken from the original Calexico parasite colonization plot (farmed by L. Shephard) and those taken thereafter from a neighboring field (farmed by R. Parker) after the grower's lease on the former field was relinquished. Since these fields were nearly adjacent to each other and under essentially the same farming practices, it was felt that reasonably similar trends occurred in both of them, and for this reason the records obtained from them were plotted in the single graph.

Coccinellid Activity.—The histogram in figure 1 traces the trends in coccinellid abundance in the Calexico plot during the year. *Hippodamia convergens* G. M. was the only species of importance encountered in the Calexico plot. Occasionally specimens of *Cycloneda sanguinea* (Linn.) and *Olla abdominalis* (Say) were collected, but these species played no significant role in the control of the aphid. From the graph it can be seen that *H. convergens* was present in the study field in greater or lesser abundance from January through August. It first became common in March when the pea aphid, *Macrosiphum pisi* (Harris), became quite abundant. The early buildup of *H. convergens* on pea aphid probably had a highly significant effect on the *T. maculata* population trend, for as the pea aphid declined in abundance the coccinellid adults and larvae shifted their attack to the former and helped prevent its build-up under the favorable climatic conditions of late spring.

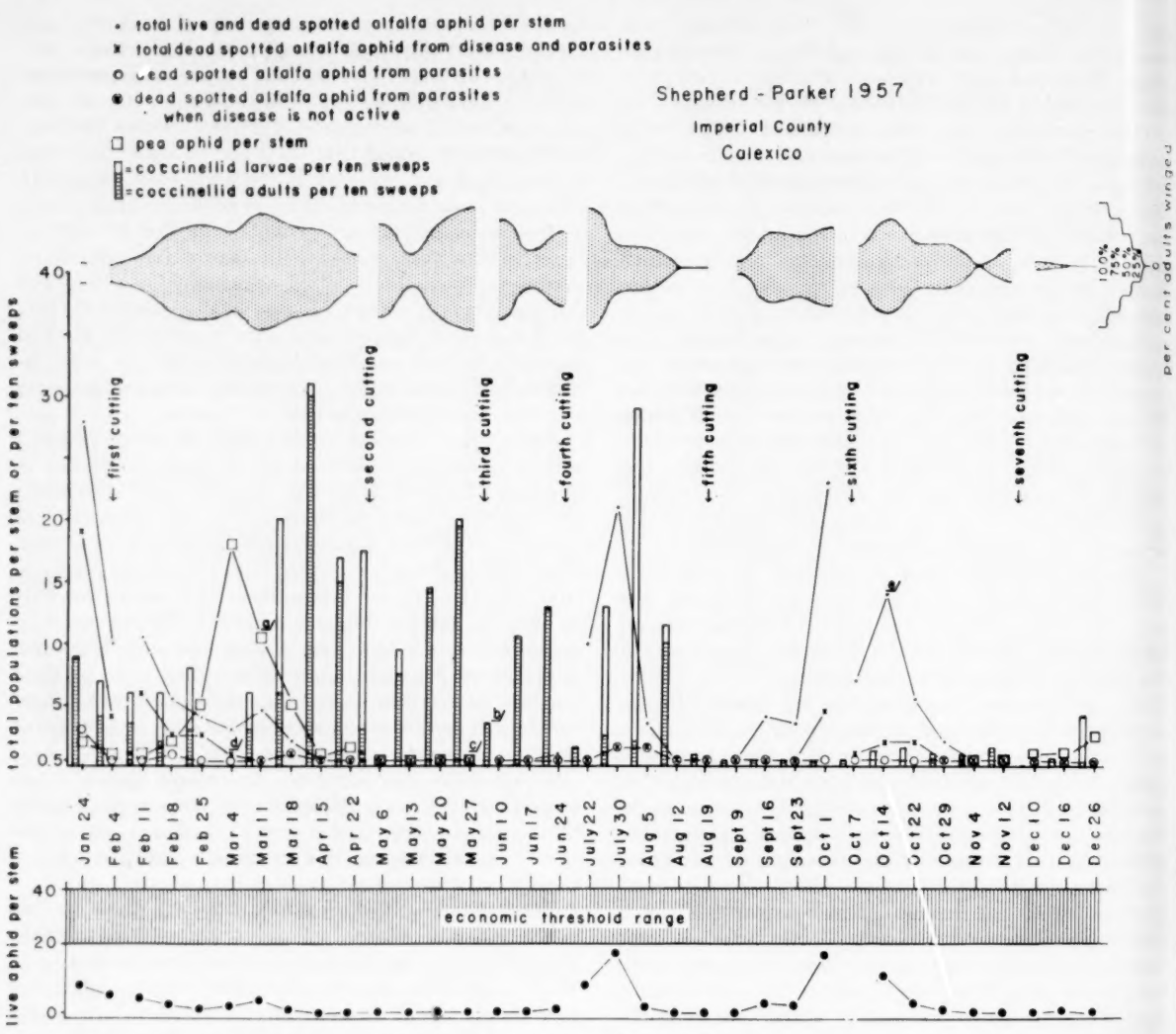
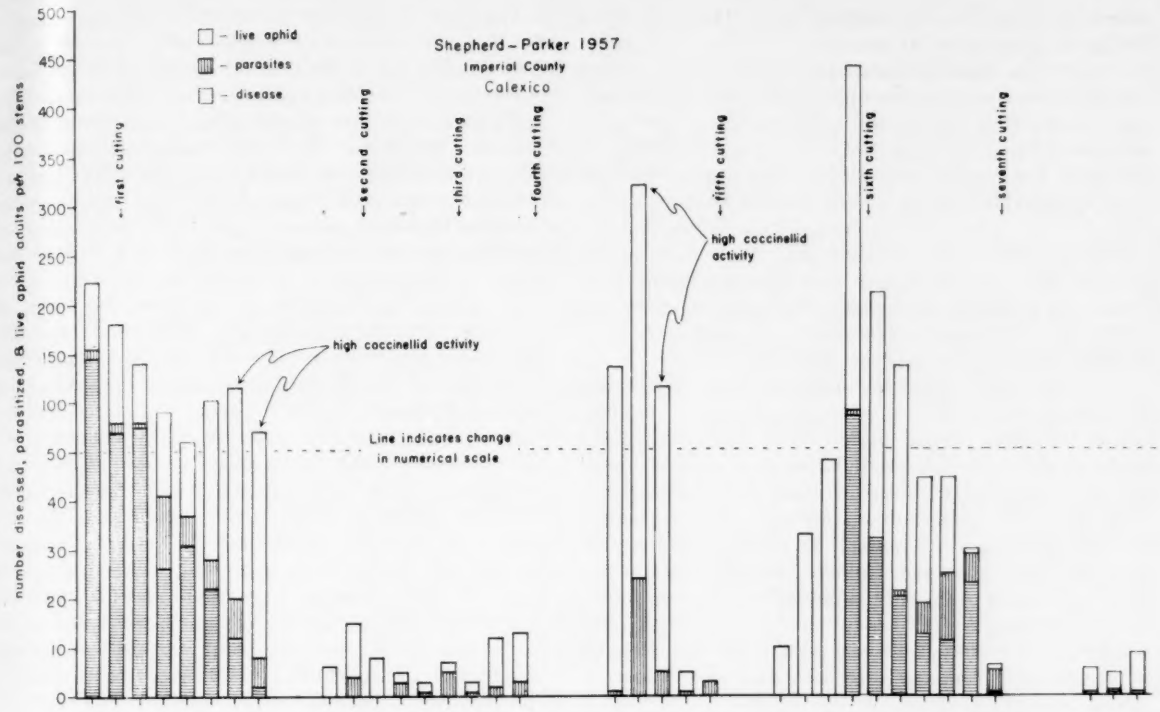


FIG. 1.—Population trends during 1957 of the spotted alfalfa aphid (*Therioaphis maculata*), the pea aphid (*Macrosiphum pisi*), and coccinellids in a commercial alfalfa field located at Calexico, California. The undulating shaded band at the top of the upper graph shows the percentages of alates in the adult *T. maculata* populations on each date. The lower graph shows the trend of live *T. maculata* in relation to the economic threshold during the year.

[a] Diseased pea aphids were first encountered on March 11 when a mortality of 50% was recorded. Mortality from disease reached a peak of 75% on March 18 and then dropped to 10% on April 15.
[b] High wind affected the coccinellid sweep samples on this date.
[c] The pea aphid was essentially absent from the field from May 27 until November 4.
[d] Cocoons of *Praon palitans* were first observed on March 4.
[e] Heavy rainfall occurred immediately prior to the sampling date.

The role of the coccinellid became especially critical in March when fungus disease, which had taken a heavy toll of the *T. maculata* population during the previous months, disappeared from the field. A second buildup of *Hippodamia convergens* occurred in July when *T. maculata* suddenly rose to threatening abundance. Close scrutiny of figure 1 reveals that this upsurge of *T. maculata* on the fourth cutting resulted from a very heavy invasion of alate aphids. Alate percentages in the aphid population are shown in the undulating shaded band running across the top of the graph. From this band it can be seen that on July 22 almost 100% of the adult aphids in the field were alates. At this time alates comprised the bulk of the entire aphid population, and since the field had lain barren of alfalfa foliage for 3 weeks between cuttings, these winged aphids could have come only from outside the field. The histogram for the period of the fourth cutting reflects the remarkably rapid response of *H. convergens* to the upsurge of *T. maculata* and the very heavy production of larvae about mid-cutting. There seems little reason to doubt that the coccinellid was largely responsible for preventing the aphid from reaching economic abundance or that it eventually decimated the population.

After the clean-up of the midsummer outbreak of *T. maculata*, *Hippodamia convergens* rapidly decreased in abundance and by early fall occurred only in very low numbers in the field. When *T. maculata* again increased in abundance in October, coccinellids failed to increase to



		Jan 24	Feb 4	Feb 11	Feb 18	Feb 25	Mar 4	Mar 11	Mar 18	Apr 15	Apr 22	May 6	May 13	May 20	May 27	Jun 10	Jun 17	Jun 24	Jul 22	Jul 30	Aug 5	Aug 12	Aug 19	Sept 9	Sept 16	Sept 23	Oct 1	Oct 7	Oct 14	Oct 22	Oct 29	Nov 4	Nov 11	Dec 10	Dec 16	Dec 26
parasite pupae per 100 stems	Praon	0	0	0	0	0	2	1	4	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Trioxys	10	9	4	15	6	4	7	2	0	3	0	2	0	4	1	2	3	–	24	5	1	3	0	0	0	–	0	1	6	14	6	4	–	1	1
	Aphelinus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
totals / 100 stems		10	9	4	15	6	6	8	6	0	4	0	2	1	4	1	2	3	1	24	5	1	3	0	0	0	1	0	1	6	14	6	4	1	1	1
% parasitizations[a]		13	9	6	30	26	8	8	9	0	27	0	56	20	64	17	13	23	1	8	4	20	100	0	0	0	1	0	1	19	40	81	80	14	29	10
% diseased aphid		66	42	54	42	64	24	11	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	20	15	15	32	35	95	43	0	2	0
adult parasite per 100 sweeps	Praon	0	0	0	0	0	–	–	7	14	4	6	1	0	0	1	0	3	1	3	1	0	0	0	0	0	1	–	0	0	0	0	0	0	0	0
	Trioxys	17	35	144	63	41	–	–	33	10	5	54	8	4	3	1	1	2	2	56	50	0	0	0	0	0	59	–	6	2	0	1	7	1	5	3
	Aphelinus	0	0	0	0	0	–	–	–	0	0	0	0	0	–	0	0	0	0	0	0	0	0	0	0	0	0	–	0	0	0	0	0	0	0	0
total / 100 sweeps		17	35	144	63	41	–	–	41	[a]	9	60	9	4	4	2	1	5	3	59	51	0	0	0	0	0	60	–	6	2	0	1	7	1	5	3

FIG. 2.—Trends during 1957 in incidence of fungus disease and parasitization of adult *Therioaphis maculata* occurring in a commercial alfalfa field at Calexico, California.

[a] Parasitization was calculated on the basis of mummified adult *T. maculata* vs. healthy adults in the stem samples. Disease was calculated on the basis of fungus-produced cadavers vs. healthy adults in the stem samples. These two mortality factors were calculated independently of each other and the mortalities are not cumulative. For example, on November 4, 95% of the total adult population was diseased while 81% of the surviving 5% of the aphids were parasitized.

any extent and apparently played no important role in its control.

Fungus Disease.—The data in figures 1 and 2 show very clearly the incidence of disease during the cooler parts of the year in the Calexico plot and its absence from the field in an infectious state from about mid-March until early October. When the evaluation studies were started in January the population of *T. maculata* was suffering very heavily from an epizootic caused by *Entomophthora exitialis*, but as the temperatures warmed and humidities declined, the incidence of disease steadily dwindled. Fungus disease completely disappeared from the aphid population by mid-April. However, the high percentages of diseased aphids recorded in January and February, correlated with the declining slope of the aphid population curve during that period, strongly indicate that *E. exitialis* had a controlling effect on *T. maculata* in the study field during the winter of 1956–57.

The development of the fall epizootic is very clearly depicted in the graphic and tabular data. The most remarkable feature of this epizootic was its very sudden appearance after a period of more than 5 months during which not a single diseased aphid was recorded from the samples. Because of the epizootic proportions of the disease outbreak at this time, it seems probable that the fungus had remained in the field in the resting-spore stage throughout the unfavorable hot, dry period. The return of favorable climatic conditions, coinciding with the fall upsurge of *T. maculata*, permitted *Entomophthora exitialis* to infect its host and reach epizootic proportions before the aphid could reach a damaging population density.

Parasite Activity.—The role of *Trioxys utilis* in the Calexico plot appears to have been much less important than that of the coccinellid or fungus disease. This is not surprising, inasmuch as *T. utilis* had barely become established throughout the study field when the evaluation program was initiated. Thus, there can be no question that during the first half of the season the mortality produced by *T. utilis* was definitely secondary to that produced by fungus disease in February and March, and to that pro-

duced by *Hippodamia convergens* later. However, even though it played a secondary role, *T. utilis* destroyed a portion of the aphid population throughout this period which had escaped disease and predation, and this in itself was important in the overall amelioration of the aphid problem in the field during the first half of 1957. Again in the fall of the year *T. utilis* played what appears to have been a secondary role to fungus disease. However, analysis of figure 2 and the tabulated data shows that the parasite destroyed an extremely high percentage of the aphids which escaped disease and increased in effectiveness as the epizootic diminished. Furthermore, at the end of the year when disease had once more waned, *T. utilis* was still actively attacking the aphid.

It would appear from the data obtained at Calexico that where coccinellids, disease and parasites are coexistent, the latter play a subtle role in which they affect that portion of the aphid population that escapes disease and predation. In fact, the true extent of parasitization is often masked by predation or epizootics, since the bulk of the aphids, parasitized as well as healthy, may be destroyed before the parasites pupate. Nevertheless, the fact that the fraction of the aphid population surviving predation and disease is often heavily parasitized is highly significant, as it is these survivors which pose the threat of reinfestation. With heavy parasitization this threat, of course, is greatly reduced.

The frequently heavy attack of *Trioxys utilis* upon low population densities of *T. maculata* would also appear to be extremely important, for at these low densities coccinellids and disease do not normally function effectively. This role of the parasite would seem to have a further dampening effect on the extreme population oscillations which so characterized the behavior of the aphid prior to 1957.

POPULATION TRENDS.—*Lancaster, California* (Figs. 3 and 4).—Lancaster is located in the Antelope Valley on the southwestern edge of the Mojave Desert at an elevation of about 2,500 feet. The climate in this area verges on a Great-Basin type, the winters being relatively cold and the summers hot but less so than those of the lower deserts of California. Long-term United States Weather Bureau records obtained at Barstow (U.S.D.A. Yearbook 1941), which is about 55 miles east of the Lancaster study plot, show an average temperature of 83.8° F. for July and 46.1° F. for January. Killing frosts occur from November 8 to March 8 according to these data. Rainfall averages about 5 inches per year at Barstow with the bulk of the precipitation falling from November through March. Actually, because of the more westerly location of Lancaster, summer temperatures are probably somewhat lower than those recorded for Barstow, and annual precipitation is probably slightly higher. Despite the relatively moderate summer climate at Lancaster humidities in the alfalfa fields are very low, apparently being influenced by the strong winds that blow almost constantly during this season. A generally sandy soil, which dries quickly following irrigation, also probably contributes to lowered humidities in the alfalfa fields.

Because of the relatively severe winter climate in the Mojave Desert, alfalfa, principally composed of the varieties California Common and Caliverde, is dormant during this period.

The study field in the Antelope Valley was located on the R. L. Reynolds ranch 8 miles east of Lancaster. This field was the site of the original successful establishment of the three *T. maculata* parasites in California, and thus had a parasite history extending back to midsummer 1956 (van den Bosch *et al.* 1959). Evaluation studies were not initiated until late February 1957, as the alfalfa had been almost completely dormant through the previous winter months. This field was not typical of those in the surrounding area for two main reasons; first, it had not been treated with insecticides for the 2 years prior to the 1957 observations; and secondly, it had come under complete biological control during the late summer and fall of 1956. Because of this latter condition *T. maculata* had become relatively scarce in the field by the end of 1956. As a consequence of this aphid scarcity the two principal parasites, *Praon palitans* and *Trioxys utilis*, also became scarce at the year's end. Fields in the area surrounding the evaluation plot had been heavily treated with phosphate insecticides throughout the 1956 growing season, and after treatment was suspended in the fall *T. maculata* rebounded quickly and heavy infestations were general late in the year. *P. palitans*, particularly, reproduced to very great abundance on these late-season, untreated aphid populations. Thus, these fields generally harbored much larger numbers of diapausing *P. palitans* during the winter than the study field. Sporadic observations in the study field during the winter months did reveal, however, considerable numbers of diapausing *P. palitans* in the ground litter. Furthermore, *T. utilis* reproduced throughout the winter on the very light aphid population that occurred on the scattered sprigs of green foliage in the alfalfa crowns. These observations also showed two coccinellid species, *Hippodamia parenthesis* (Say) and *H. sinuata* Mulsant, as well as a number of other predator species, to be hibernating or otherwise overwintering in the ground litter or in the crowns of the alfalfa plants. Therefore, despite the relatively low *T. maculata* and beneficial insect populations of late 1956 and severe winter conditions which included a heavy snowfall and a minimum temperature of about 8° F. on January 29, the aphid, as well as *P. palitans*, *T. utilis*, and several important predator species, survived this hardship period in the study field.

Perhaps the most striking feature of the Lancaster data was the complete absence of any entomophthoraceous fungus diseases of *T. maculata* from the study field during the entire alfalfa-growing season. This reflects a condition that is general throughout the Mojave Desert area and unquestionably relates to the unfavorable humidity conditions that prevail in this arid, windy region.

A striking difference in the relative importance of *Praon palitans* and *Trioxys utilis*, as compared with the Calexico plot, is also reflected by the Lancaster data. Thus, whereas *T. utilis* predominated in the ratio of 15.7:1 in the mummy counts and 14.6:1 in the sweepings at Calexico, it was secondary to *P. palitans* at Lancaster in the ratio of 1:99.9 in the mummy counts and 1:4.7 in the sweepings. The wide discrepancy between the mummy ratios and the sweep ratios at Lancaster is not fully understood. However, since the bulk of the *T. utilis* was swept during the first 2 months of the survey, it seems probable that these adults represented a residue of the population which passed through the winter. Further-

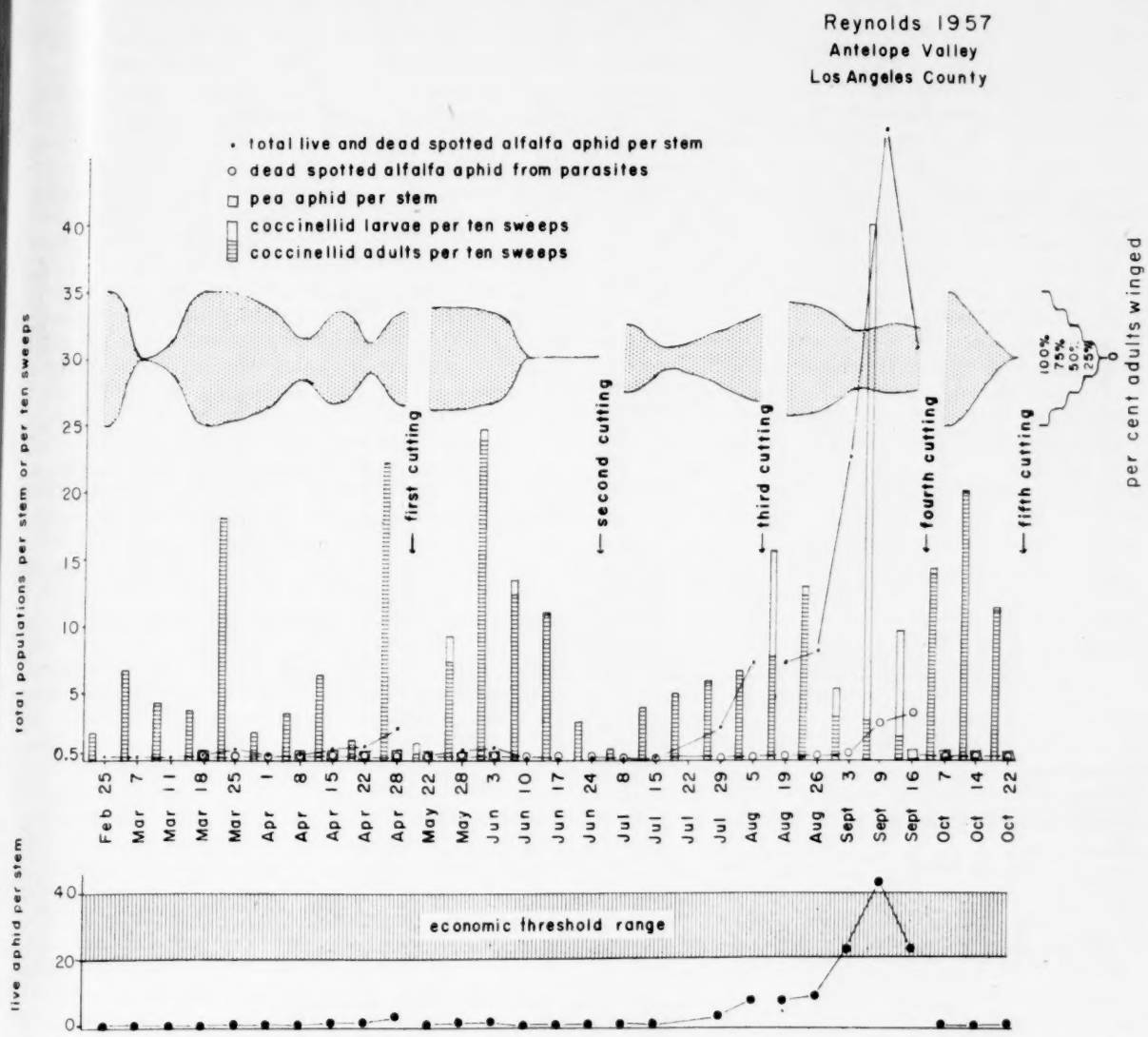
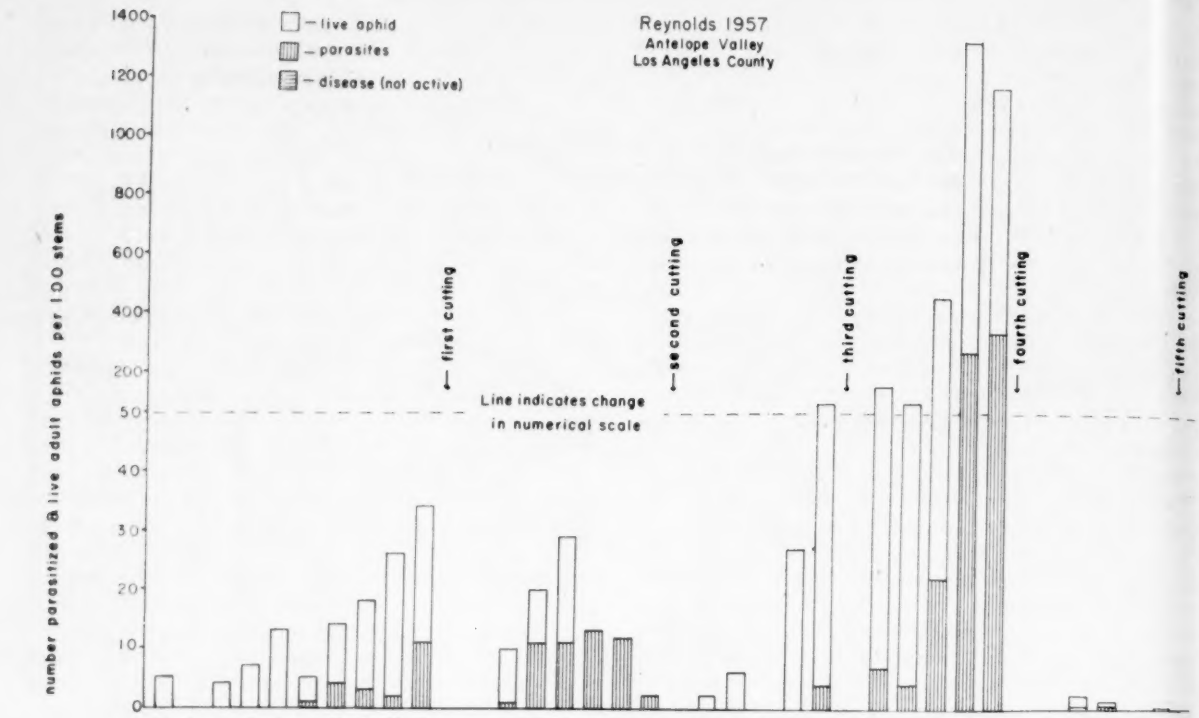


FIG. 3.—Population trends during 1957 of the spotted alfalfa aphid (*Therioaphis maculata*), the pea aphid (*Macrosiphum pisi*), and coccinellids in a commercial alfalfa field located eight miles east of Lancaster, California. The undulating shaded band at the top of the upper graph shows the percentages of alates in the adult *T. maculata* populations on each date. The lower graph shows the trend of live *T. maculata* in relation to the economic threshold during the year.

more, the strong winds that blow almost daily during summer in the Antelope Valley could very well have dislodged a significant proportion of the *T. utilis* mummies from the alfalfa plants, and these, of course, would not have appeared in the samples. A third possibility is that *T. utilis* adults at times migrated into the field but because of cutting or competition from coccinellids never produced significant parasitization.

At Lancaster, as at Calexico, the study field was essentially free of a severe aphid infestation throughout the 1957 growing season. No insecticide was applied during the year. The infestation which developed in September did reach economic proportions, but it barely exceeded the economic threshold range before biotic factors (principally coccinellids) brought it under control. The population trend for *T. maculata* in this evaluation field approximated that of the aphid for the entire east side of the Antelope Valley in 1957. In the broader area, however, populations in the spring appeared to be considerably higher than those in the study plot, though not generally of economic proportions. Aphids in these fields were very heavily attacked by *Praon palitans*, enormous numbers of which diapaused during the winter following their heavy build-up in the fall of 1956. The reason for the slow build-up of *T. maculata* in the evaluation field, as well as over the entire eastern Antelope Valley in 1957, appears to have been related to the mortality effected by parasites and to a lesser degree by coccinellid adults.

Coccinellid Activity.—Coccinellids occur as a complex of species in the Antelope Valley, with at least four species, *Hippodamia convergens*, *H. quinquesignata punctulata* Le Conte, *H. sinuata*, and *H. parenthesis*, playing important roles. Of these the last-named was predominant in the evaluation plot during the year. *Olla abdominalis* and



		Feb 25	Mar 7	Mar 11	Mar 18	Mar 25	Apr 1	Apr 8	Apr 15	Apr 22	Apr 28	May 22	May 28	Jun 3	Jun 10	Jun 17	Jun 24	July 8	July 15	July 22	July 29	Aug 5	Aug 19	Aug 26	Sept 3	Sept 9	Sept 16	Oct 7	Oct 14	Oct 22
parasite pupae per 100 stems	Praon	0	0	0	0	0	1	4	3	2	9	1	8	11	12	12	2	0	0		0	3	7	4	22	263	332	2	1	0
	Trioxys	0	0	0	0	0	0	0	0	0	2	0	3	0	1	0	0	0	0		0	1	0	0	0	0	0	0	0	0
	Aphelinus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0
totals / 100 stems		0	0	0	0	0	1	4	3	2	11	1	11	11	13	12	2	0	0		0	4	7	4	22	263	332	2	1	0
% parasitizations		0	0	0	0	0	20	29	16	8	32	10	55	38	100	100	100	0	0		0	5	5	4	5	20	29	21	10	0
% diseased aphid		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
adult parasite per 100 sweeps	Praon	15	60	41	52	81	14	52	35	3	117	16	70	36	11	3	0	0	1	1	1	1	1	3	7	44	211	53	2	0
	Trioxys	12	27	12	2	6	7	14	5	0	26	1	9	7	2	0	0	0	1	1	0	5	4	0	0	4	46	8	0	1
	Aphelinus	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	1
totals / 100 sweeps		27	87	53	54	87	21	67	40	3	143	17	79	43	13	3	0	1	2	2	1	6	5	3	7	48	257	62	2	2

FIG. 4.—Trend during 1957 of parasitization of adult *Therioaphis maculata* occurring in a commercial alfalfa field at Lancaster, California.

[a] Parasitization was calculated on the basis of mummified adult *T. maculata* vs. healthy adults in the stem samples.

Coccinella novemnotata franciscana Casey also occurred in the field at times. Because of the scarcity of aphids in the plot during the spring, there was essentially no coccinellid reproduction during this period. In fact, the only larvae that were encountered before late summer were the few that were produced in late May and early June following a slight rise in aphid abundance. This is reflected by the white portions of the histogram in figure 3. Despite the limited reproduction of coccinellids during the spring, their influence on the *T. maculata* population density must have been profound, particularly in the early spring when relatively large numbers of hungry adult beetles emerged from hibernation and invaded the field. The histogram in figure 3 shows that coccinellid activity dropped to a low ebb for a period of about a month in midsummer (midJune to midJuly). However, the beetles quickly responded to the upsurge of *T. maculata* in late July as is evidenced by the high proportion of larvae recorded from the August 19 sweepings. The invasion of the plot by large numbers of alate *T. maculata* following the third cutting permitted the aphid to increase in abundance despite heavy predation. Nevertheless, an extremely heavy population of coccinellid larvae (as indicated by the sweepings of September 9) quickly brought the infestation under control, and predation by adult beetles following the fourth cutting completely decimated the population.

Parasite Activity.—The first parasite-mummified aphids did not appear in the samples at Lancaster until April 1, although adult *Praon palitans* had been active for at least 5 weeks prior to this date (as indicated by the sweep data in the tabular portion of figure 4). The lag in appearance of mummies in the samples is probably primarily accounted for by the protracted developmental period of *P. palitans* within the parasitized aphid hosts during this cool time of the year. A maximum parasitization rate of 32% was recorded on the first cutting on April 28. This parasitization, combined with coccinellid predation, was

apparently sufficient to prevent any explosive increase in the aphid population density. On the second cutting parasitization reached a very high level approaching 100% on three successive sampling dates. There can be little question that at this time *P. palitans* played a highly significant role in the biological control of *T. maculata*. Following this period *P. palitans* became very scarce in the field, particularly as the aphid host dropped to very low abundance. Unquestionably, the parasite abundance was also further affected by the 2-week alfalfa-free period between the second and third cuttings. During the latter portion of the second cutting and the entire third cutting, adult *P. palitans* were only rarely swept in the field. This is reflected in the tabular data for parasite sweepings in figure 4. Thus, the period from about midJune to midJuly represented a period of scarcity of parasites as well as coccinellids. At this time *T. maculata* began to increase in abundance, probably because of its temporary release from the two major biotic mortality factors. *P. palitans* showed its first response to this increase on August 5, when 5% parasitization was recorded following 3 weeks of zero recordings. On the first sampling date of the fourth cutting, the *T. maculata* population averaged approximately four aphids per stem. Significantly, this population was composed almost entirely of alates, indicating an invasion of winged forms from surrounding fields. On the same date only a single *P. palitans* was recorded from 100 net sweepings. This proved to be a very unfavorable host-parasite ratio, especially since the aphid population was entirely comprised of reproducing adults capable of increasing their numbers manyfold within a relatively short period of time. The rapid rise of the aphid population density on the fourth cutting indicates that this is precisely what happened. Considering these circumstances the response of *P. palitans* was quite remarkable, for within a period of 28 days parasitization percentage increased 6-fold (from 5 to 29%) and the abundance of adult *P. palitans* over 200-fold (from 1 per 100 sweeps to 211 per 100 sweeps). Furthermore, by destroying somewhere between 20 and 29% of the adult aphids by midcutting, *P. palitans* unquestionably lessened the severity of the infestation which otherwise would have exceeded the economic threshold range to a much greater degree than actually was the case.

Trioxys utilis mummified aphids were not encountered at any time during the outbreak on the fourth cutting. However, the sweeping of considerable numbers of adults of this species on September 16 indicates that it was much more active than indicated by the stem counts. As mentioned earlier, *T. utilis* mummies are very weakly attached to the alfalfa plants, and the strong winds that prevail in the area of the study plot could well have stripped the bulk of these from the plants so that they were missed in the sampling. If this were the case, the ratio of 46 *T. utilis* adults to 211 *Praon palitans* adults in the September 16 sweepings would indicate a possible parasitization of 5 or 6% by the former, which, when added to the 29% recorded for *P. palitans*, would bring the maximum parasitization rate on this particular infestation up to approximately 35%.

On the fifth cutting the *T. maculata* population density in the Lancaster study plot reverted to a very low level. Both *Praon palitans* and *Trioxys utilis* became extremely scarce as a result of the low aphid density and the protracted alfalfa-free period between the fourth and fifth cuttings. Cold weather ensued following the harvest of the fifth cutting, the alfalfa became dormant, and sampling was discontinued for the year.

POPULATION TRENDS.—*Cawelo, Kern County, California* (Figs. 5 and 6).—The Cawelo plot was located on the W. B. Camp Ranch about 14 miles north of Bakersfield in the southern San Joaquin Valley. Long-term United States Weather Bureau data obtained at Bakersfield (U.S.D.A. Yearbook 1941) show the average temperature for July to be 83.5° F. and that for January to be 47.0° F. This would indicate a climate quite similar to that of the Antelope Valley. However, the average temperature for January is somewhat deceiving in that it is influenced by dense ground fogs (tule fogs) which sometimes are of several weeks' duration. During these foggy periods diurnal temperatures fluctuate only slightly, ranging from the high 30's at night to the low 40's during the day. Unquestionably these protracted foggy periods greatly influence the average temperature during January. At Bakersfield killing frosts occur between November 25 and February 21, a period 1 month shorter than the period of killing frosts at Barstow. Average annual rainfall at Bakersfield is slightly over 6 inches. Essentially all precipitation occurs from November through April.

Both the California Common and Caliverde alfalfa varieties are widely grown in the San Joaquin Valley. Alfalfa does not become fully dormant in this area but, because of the depressed winter temperatures, develops extremely slowly after it has been cut for the last time or grazed off by sheep. The alfalfa in the Cawelo plot was California Common.

Sampling was commenced on February 26 at a time when the first cutting of alfalfa was about one-third grown. The mowing practice in this field was different from that in most commercial fields, including the Calexico and Lancaster study plots, in that it was on a strip-cutting cycle. Under this system alternate double irrigation beds were placed off cycle with each other by about 2 weeks so that at all times strips of standing alfalfa occurred in the field. Sampling was done in alfalfa of only one of the cutting cycles.

Coccinellid Activity.—Coccinellids unquestionably played the predominant role in the natural control of *T. maculata* in the Cawelo evaluation field. The coccinellid population at Cawelo was comprised principally of two species, *Hippodamia convergens* and *H. quinquesignata punctulata*, which were of about equal importance over the season. At the time sampling was begun, adult beetles were already present in the field. The extent of their activity during March and early April is somewhat obscure because of a disrupted sampling schedule. However, on April 15 an upward trend in abundance of the pea aphid was recorded, and on this date coccinellid larvae were collected, indicating that sufficient nutrition had been available to the adults to induce oögenesis and oviposition. During the following 3 weeks the pea aphid population density continued its upward trend, and correlated with this was an increase in the population density of *T. maculata*. The increase in abundance of *T. maculata* possibly occurred as a result of its release from predation pressure as the coccinellids shifted their attack to the

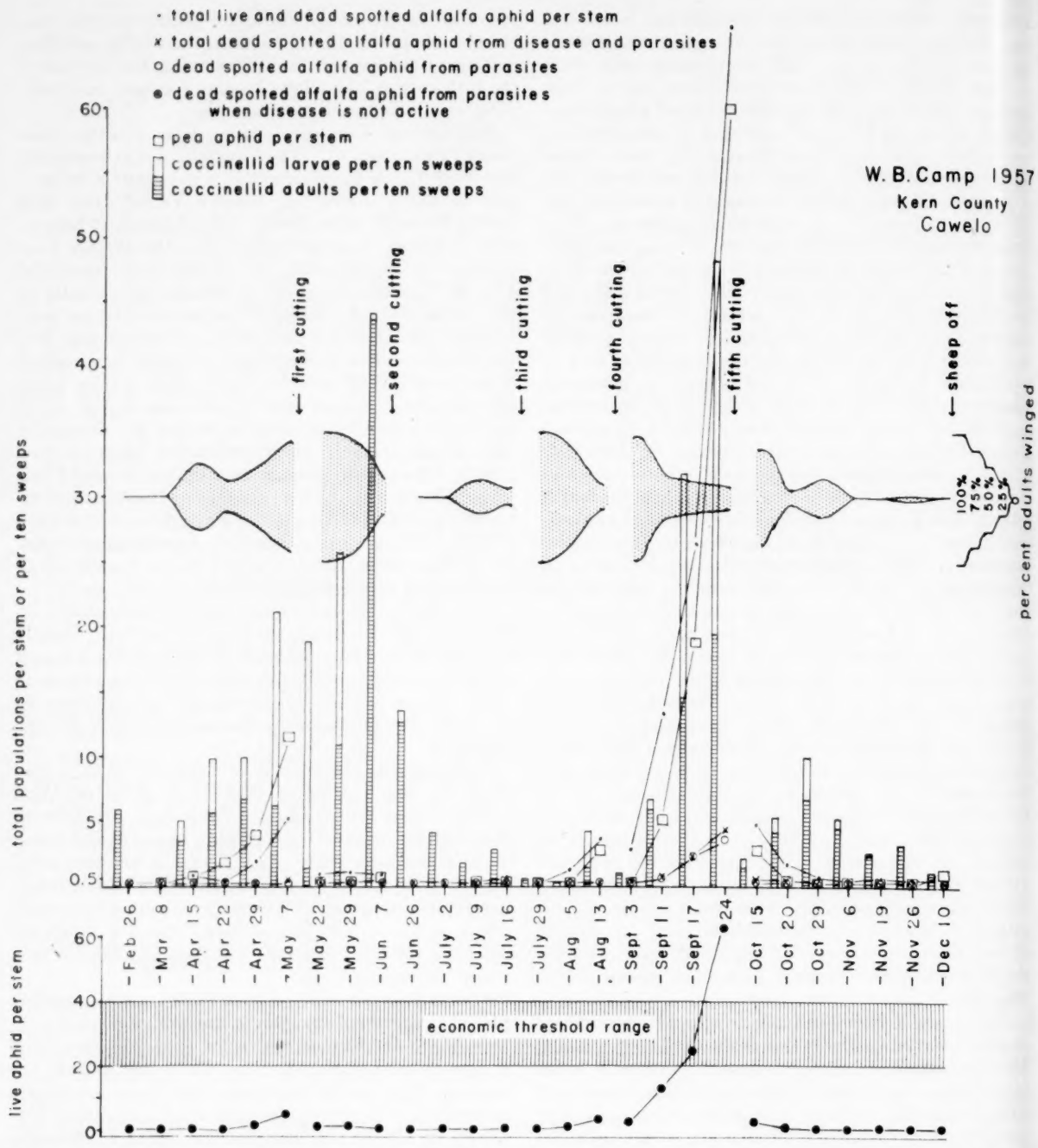
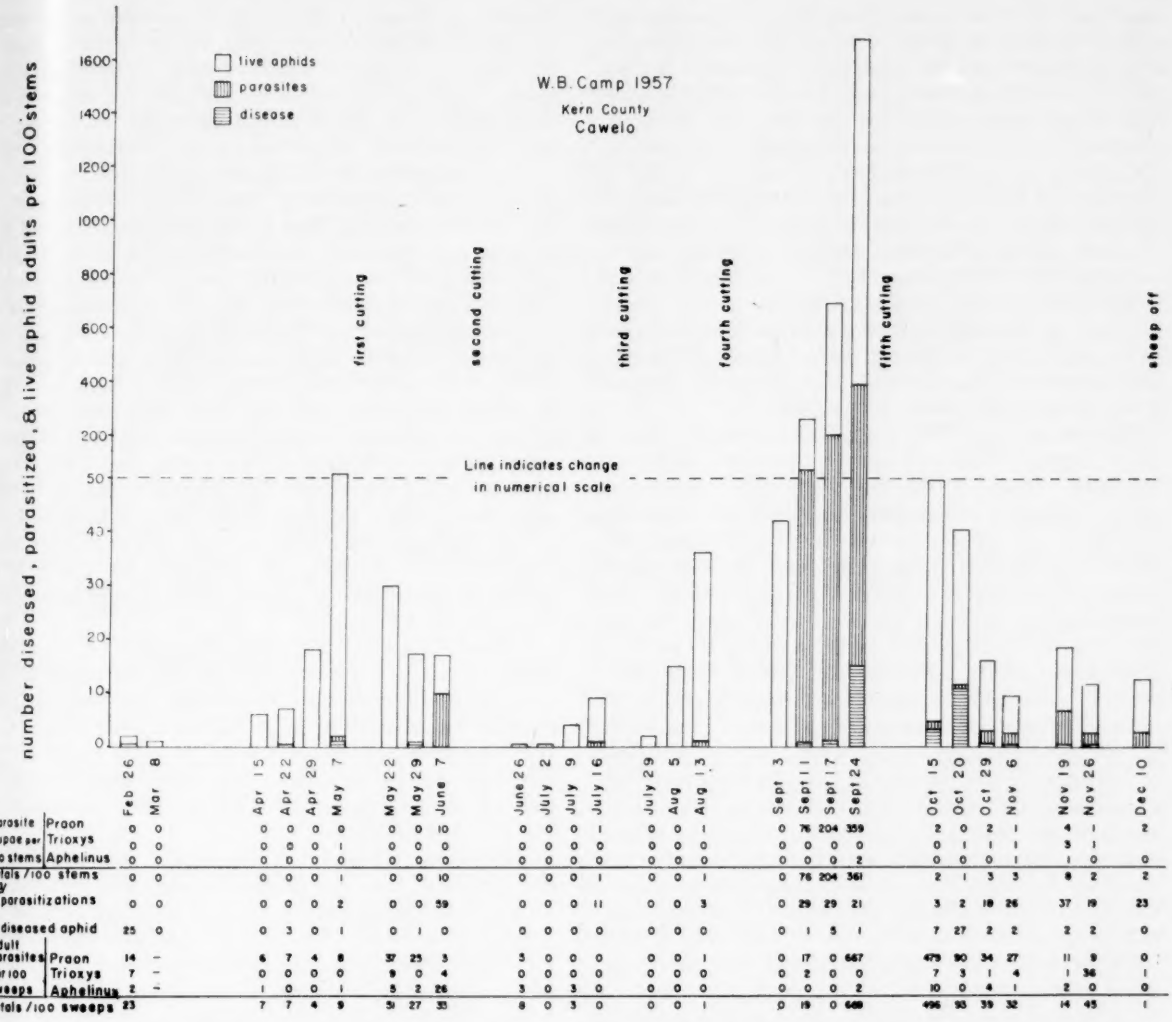


FIG. 5.—Population trends during 1957 of the spotted alfalfa aphid (*Therioaphis maculata*), the pea aphid (*Macrosiphum pisi*), and coccinellids in a commercial alfalfa field located at Cawelo, California. The undulating shaded band at the top of the upper graph shows the percentages of alates in the adult *T. maculata* populations on each date. The lower graph shows the trend of live *T. maculata* in relation to the economic threshold during the year.

more abundant pea aphid. Neither aphid species reached a serious infestation level by the time the cutting was harvested, and in the meantime heavy coccinellid reproduction occurred. By the beginning of the second cutting the population densities of both *M. pisi* and *T. maculata* were once more reduced to low levels. Unquestionably the harvesting process adversely affected both species, but it is also apparent from the heavy coccinellid build-up that predation helped greatly to reduce the aphid population densities. Following this reproductive activity during the first and second cuttings, coccinellids rapidly decreased in abundance, and during mid-summer (*i.e.*, late third cutting and early fourth cutting), they were extremely scarce.

Late in the fourth cutting both *T. maculata* and *M. pisi* once more began to increase in abundance. Coccinel-



		Feb 26	Mar 8	Apr 15	Apr 22	Apr 29	May 7	May 22	May 29	June 7	June 26	July 2	July 9	July 16	July 29	Aug 5	Aug 13	Sept 3	Sept 11	Sept 17	Sept 24	Oct 15	Oct 20	Oct 29	Nov 6	Nov 19	Nov 26	Dec 10
parasite pupae per 100 stems	Praon	0	0	0	0	0	0	0	0	10	0	0	0	1	0	0	1	0	76	204	359	2	0	2	1	4	–	2
	Trioxys	0	0	0	0	0	–	0	0	0	0	0	0	–	0	0	–	0	0	0	0	0	1	1	1	3	1	
	Aphelinus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	1	1	0	0
Totals /100 stems		0	0	0	0	0	1	0	0	10	0	0	0	1	0	0	1	0	76	204	361	2	1	3	3	8	2	2
% parasitizations[a]		0	0	0	0	0	2	0	0	59	0	0	0	11	0	0	3	0	29	29	21	3	2	18	26	37	19	23
% diseased aphid		25	0	0	3	0	1	0	1	0	0	0	0	0	0	0	0	0	1	5	1	7	27	2	2	2	2	0
adult parasites per 100 sweeps	Praon	14	–	6	7	4	8	37	25	3	3	0	0	0	0	0	1	0	17	0	667	479	90	34	27	11	9	0
	Trioxys	7	–	0	0	0	0	9	0	4	0	0	0	0	0	0	0	0	2	0	0	7	3	1	4	1	36	1
	Aphelinus	2	–	1	0	0	1	5	2	26	3	0	3	0	0	0	0	0	0	0	2	10	0	4	1	2	0	0
totals /100 sweeps		23		7	7	4	9	51	27	33	8	0	3	0	0	0	1	0	19	0	669	496	93	39	32	14	45	1

FIG. 6.—Trends during 1957 in incidence of fungus disease and parasitization of adult *Therioaphis maculata* occurring in a commercial alfalfa field at Cawelo, California.

[a] Parasitization was calculated on the basis of mummified adult *T. maculata* vs. healthy adults in the stem samples. Disease was calculated on the basis of fungus-produced cadavers vs. healthy adults in the stem samples. These two mortality factors were calculated independently of each other and the mortalities are not cumulative.

lids, though scarce, responded very quickly as indicated by the presence of larvae in the August 13 samples. These trends were interrupted by the harvesting of the fourth alfalfa cutting but resumed again at the beginning of the fifth cutting. However, as had happened at Calexico and Lancaster, a heavy migration of alate *T. maculata* gave impetus to the build-up of this species and it very quickly rose to economic abundance. *M. pisi* also increased in abundance, but its rise was slower than that of *T. maculata*, as it had started from a much lower initial population density at the beginning of the cutting. The response of the coccinellids to the increase in aphid abundance was very rapid. Not only was there a heavy production of larvae, but apparently there was also an invasion of the sampling area by adult beetles from the adjoining alfalfa as evidenced by a steady increase of adults in the sweepings.

It is noteworthy that the increases in abundance of *T. maculata* in both spring and late summer were correlated with upsurges of the pea aphid. In the spring the increase in abundance of *M. pisi* appears to have been beneficial, for it provided sufficient nutrition to induce oögenesis and oviposition in the coccinellid adults that had emerged from hibernation. These beetles and their progeny, in turn, appear to have had a suppressive effect on *T. maculata* through mid-summer. In the fall, however, pea aphid population may have acted as a buffer to coccinellid predation on *T. maculata*. Thus, it is possible that if the pea aphid had not been present at this time the coccinellids would have been forced to concentrate their attack on *T. maculata* and might possibly have prevented the latter from reaching economic abundance or at least reduced the severity of the infestation.

Fungus Disease.—Fungus disease was active at the time observations were initiated in February, affecting about 25% of the aphid population. However, despite a

significant rise in the aphid infestation in late spring, only a slight incidence of disease was recorded, and thereafter it disappeared from the field until September. In September, when *T. maculata* surged to economic abundance, disease did appear again for the first time in over 3 months; however, its effect on the population was negligible, causing a maximum mortality of only 5%. In the fall, as conditions more favorable to its development occurred, disease reached considerably higher incidence on a rather moderate aphid infestation. Maximum disease was recorded on October 20 when 27% of the aphids were affected. Slight disease incidence was recorded in November, but on the final sampling date no infected aphids were encountered. In general, fungus disease appeared to play a relatively minor role in the biological control of *T. maculata* in the Cawelo study field.

Parasite Activity.—The parasitization trend at Cawelo was considerably more erratic than that at Calexico and Lancaster. However, the trend generally followed the pattern observed at Lancaster, excepting that *Aphelinus semiflavus* was present in the plot to a considerably greater degree than at Lancaster and, for that matter, Calexico. Over the season *Praon palitans* was the overwhelmingly predominant parasite, occurring in the ratio of 19.3:1 over *Trioxys utilis*, and 23.3:1 over *A. semiflavus* in the sweep counts, and in the ratio of 82.9:1 and 165.8:1 over *T. utilis* and *A. semiflavus*, respectively, in the mummy counts. The recovery of considerable numbers of *A. semiflavus* adults from the sweeps and the occurrence of mummies of this parasite in the plot on several occasions is of special interest. The rather heavy collection of adults of this species during the second cutting without observing any mummies in the stem counts is somewhat puzzling. However, it is possible that these adults had moved into the evaluation area from the off-cycle alternate strips when these were cut. *T. utilis* was recorded from the plot in somewhat greater numbers than *A. semiflavus*, but its role was not of any great importance. All *T. utilis* were observed either in late spring or in the fall. The absence of this species from the samples during summer is not thought to be an indication that it passed this period in aestivation, for its occurrence throughout the summer at Calexico and Lancaster would seem to preclude this. It is felt rather that *T. utilis* became so scarce in the study area that the sampling methods used simply were not sensitive enough to detect its presence. Circumstantially, it would appear that under the conditions prevailing in the Cawelo field, as well as the one at Lancaster, during 1957, *T. utilis* was for some unknown reason at a competitive disadvantage with *P. palitans* and may even have been inhibited in its activity by the latter when it became abundant.

As indicated earlier, the seasonal parasitization trend at Cawelo followed to a certain degree the pattern recorded from Lancaster. Thus, an early peak was reached on June 7 when a figure of 59% parasitization was recorded. This occurred after the incipient spring build-up of *T. maculata* had been arrested by coccinellids. Parasite activity on the low aphid populations occurring in the mid-summer alfalfa cuttings was not great, with maximums of 11% parasitization and 3% parasitization being recorded on the third and fourth cuttings, respectively. However, the response of the parasites (essentially *Praon palitans*) to the sharp upsurge of *T. maculata* on the fifth cutting was rather striking. Thus, where neither mummified aphids nor parasite adults could be collected on September 3 and parasitization for the date was recorded as zero, a maximum rate of parasitization for the cutting of 29% was attained just 8 days later. Furthermore, 669 adult parasites and 361 mummified aphids per 100 sweeps and 100 stems, respectively, were recorded on September 24, the last sampling date of the cutting. It should be pointed out that large numbers of parasitized winged aphids invaded the plot during the period between September 3 and September 11, and that mummified alate aphids contributed heavily to the parasitization rate of 29% recorded for the latter date. On September 17 a parasitization rate of 29% was also recorded. A week later the figure had dropped to 21%. However, since parasitization is based on the ratio of mummified adult aphids to healthy adult aphids, this September 24 recording may have taken place in the lag period when parasites were largely in the egg or larval stages within the aphid hosts. The harvesting of the cutting between September 24 and October 1, the next scheduled sampling date, precluded the taking of a sample which might have revealed the parasitization effected by the F_1 females.

In September at Cawelo, as at Lancaster, the parasites were not able to prevent a damaging outbreak of *T. maculata* when, after they had become extremely scarce, the field was invaded by heavy flights of alate aphids. On the other hand, parasites destroyed nearly one-third of the adult aphids by midcutting, which unquestionably prevented the infestation from reaching considerably higher levels than it did.

Therioaphis maculata remained at a low population density throughout the protracted sixth cutting, during which period parasitization reached a maximum level of 37%. It is noteworthy that all three parasite species were recovered during this cutting from both the sweep and stem samples. *Trioxys utilis*, in particular, played a very strong secondary role to *Praon palitans* as increasing numbers of this latter species passed into diapause.

DISCUSSION.—The 1957 studies just described, although carried out in only three alfalfa fields in three distinct climatic areas, have nevertheless provided considerable insight into the current and potential roles of imported parasites, as well as the roles of fungus disease and native coccinellids, in the biological control of *T. maculata*.

These studies clearly revealed that *T. maculata* was under very heavy biotic pressure during all or most of the year in each of the three fields. Furthermore, as evidenced by the reduction from previous years in severity of the spotted alfalfa aphid problem in each of the areas concerned, it would seem that this biotic pressure was general. With parasites approaching equilibrium in all three areas, as well as gaining on that position in other parts of California, and with both coccinellids and disease having apparently reached this status some time ago, there would seem to be little reason to doubt that the favorable degree of biological control which occurred in 1957 should not characterize the spotted alfalfa aphid situation in southern California in the future. This situation could possibly be drastically altered through the wide-scale destruction of parasites and coccinellids by heavy insecticide pro-

grams such as existed in 1955 and 1956. However, since a selective emergency chemical control has been perfected (Stern *et al.* 1958) and growers have become increasingly aware of the advantages of an integrated chemical and biological control program, this possibility seems remote.

In so far as degree of parasitization is concerned, both *Praon palitans* and *Trioxys utilis* demonstrated the ability to destroy high percentages of *T. maculata* populations in each area at certain times during 1957.

In addition to a depressive effect on the population density of *T. maculata*, the value of the parasites would appear to lie in their ability to dampen the oscillations of the aphid populations by (1) causing relatively heavy mortality at very low population densities when otherwise the populations might quickly rebound following release from predation, disease or adverse climatic conditions, and (2) at heavier densities by causing additional mortality over and above that caused by predators or disease alone, which has the effect of "knocking the top" off infestations that might otherwise attain or greatly exceed the economic threshold. In situations of the latter type, parasitization of only 25% or so in a given field may actually be sufficient to keep the aphid from reaching economic abundance. The high economic threshold of *T. maculata* on alfalfa hay (20 to 40 aphids per stem) provides the "working margin" that makes this possible.

Even where insecticides are used, they may at times act to enhance the effectiveness of the parasites. This relates to the ability of cocooned parasites to survive treatment with even the most toxic phosphate materials now used for aphid control (Stern *et al.* 1958). Thus, if materials were to be applied when the parasites were largely in the cocoon stage and residues did not exceed several days' duration, chemical treatment might actually shift the parasite-host ratio greatly in favor of the former. Under this condition aphids surviving treatment would be subject to heavy parasite attack and therefore greatly hindered in their ability to cause rapid reinfestation of the treated fields.

There appear to be two important situations that adversely influence the potential effectiveness of the parasites. The first of these, the periodic mowing of the alfalfa, apparently has an extremely disruptive effect on the parasite populations. This is most critical during the hotter times of the year when the parasites are exposed directly to the radiant heat of the sun and to extremely unfavorable humidity conditions between mowing time and the regrowth of the next cutting of alfalfa. The second adverse factor is the periodic invasion of the alfalfa fields by alate aphids. As indicated by the 1957 data, this phenomenon commonly occurred in fields producing regrowth after harvest. Where these invasions were heavy the parasites were literally swamped and could not, by themselves, react quickly enough to prevent economic infestations. Thus, on occasion in the study fields control had to be effected by other factors, namely, disease and coccinellids. Unquestionably this same condition developed in numerous fields in each alfalfa-growing area during the year. Some of these fields required chemical treatment. It is possible that as the full potential effect of the parasites is reached and is added to the mortality caused by predators and disease in the various alfalfa-growing areas, the intensity of alate immigration will diminish.

In some respects the most interesting phenomenon revealed in the 1957 observations was the relative success and predominance of *Praon palitans* at Lancaster (Antelope Valley) and Cawelo (San Joaquin Valley), and that of *Trioxys utilis* at Calexico (Imperial Valley). This is felt to reflect the climatic differences between the former two areas and the Imperial Valley, and the adaptation of each parasite species to each area. Thus, at Lancaster and Cawelo, where winters are considerably colder than at Calexico and alfalfa becomes fully dormant or grows only very slowly, *P. palitans* is ideally adapted because it passes this hardship period in diapause, an ability which *T. utilis* does not seem to possess (Schlinger & Hall 1959). The latter does survive the winter in both areas, but its numbers become so diminished on the low overwintering aphid populations that it takes practically the full growing season to gain reproductive momentum, and then by the time this momentum is attained it is suddenly disrupted as cold weather arrives again. Under the mild winter conditions at Calexico, on the other hand, *T. maculata* and *Trioxys utilis* reproduced continuously, while *P. palitans* spent much of the period in diapause. This enabled *T. utilis* to gain reproductive momentum in this area. Furthermore, very limited data indicate that *P. palitans* suffered heavy mortality in the pupal stage during the extremely hot summer period in the Imperial Valley while *T. utilis* did not.

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A Synopsis of the Biologies of Three Imported Parasites[1] of the Spotted Alfalfa Aphid[2]

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ABSTRACT

General observations are reported on the biologies of three imported spotted alfalfa aphid parasites (Hymenoptera: Braconidae and Eulophidae): *Praon palitans* Muesebeck, *Trioxys utilis* Muesebeck, and *Aphelinus semiflavus* Howard. Comparative life histories and such factors as mating, reproduction, oviposition, host preferences, adult longevities, sex ratios, hosts, and hyperparasites are discussed. Facultative diapause occurs in *P. palitans*, a condition believed to be previously unknown among the Aphidiinae. Uniparental and biparental reproduction was noted in *A. semiflavus*.

Soon after the presence of the spotted alfalfa aphid, *Therioaphis maculata* (Buckton), was noted in California, the Department of Biological Control of the University of California and the U. S. Department of Agriculture, Entomology Research Division, sent men into the field in an attempt to obtain parasites and predators of this new pest for introduction into the United States. In 1957 van den Bosch reported the collection of several parasites in the Middle East, and van den Bosch *et al.* (1957) reported the actual establishment and spread of these parasites in California. Hagen *et al.* (1958) have recently discussed the parasite situation more fully for northern California.

Two of the imported species, *Praon palitans* Muesebeck and *Trioxys utilis* Muesebeck, were new species that were subsequently described by Muesebeck (1956); the third species, *Aphelinus semiflavus* Howard, is considered by some to be identical with the native American form.

As these parasites have become widespread in California and their establishment has been effected in other states (Arizona, Nevada, and New Mexico), it is felt that a synopsis of their biologies might be useful to those concerned with the biology and control of the spotted alfalfa aphid. A more comprehensive biological study of each parasite is planned for publication later.

GENERAL CONSIDERATIONS.—*Praon palitans* and *Trioxys utilis* are of the subfamily Aphidiinae of the Braconidae, all species of which are solitary, internal parasites of aphids. *Aphelinus semiflavus* belongs to the subfamily Aphelininae of the Eulophidae. Members of this subfamily are parasites of aphids, scales, and whiteflies; however, apparently all species of the genus *Aphelinus* (*sensu stricto*) are internal parasites of aphids. Although both groups of parasites are solitary internal parasites of aphids, their phylogenetic distinction shows up plainly in many of their biological characteristics.

Notes pertaining to the biologies of these species have been published by van den Bosch (1956), van den Bosch *et al.* (1957), and Davis *et al.* (1957), while an extensive publication on the native form of *A. semiflavus* is the work of Hartley (1922). No other species of parasites have yet been found to attack the spotted alfalfa aphid in California, with the possible exception of the native form of *A. semiflavus* in northern California.

All laboratory biological observations were made under seemingly optimum conditions: temperature, 72° ± 5° F. and relative humidity (R.H.) 50 ± 10%, as measured by Lasico recording hygrothermographs.

PARASITE BIOLOGIES.—*Praon palitans.*—After the female parasite emerges from the pupal cocoon or "mummy," she spends a premating period of several hours cleaning herself, eating honey, and walking or flying about. Mating can then take place, and usually lasts from 10 to 25 seconds, after which the female is ready to oviposit. If mating does not take place within a day after emergence, normal oviposition occurs but the progeny are all males.

The egg is laid internally, usually in the abdomen of the host. In ovipositing, the female parasite bends her abdomen beneath the thorax and extends the ovipositor far out in front of her head to contact the aphid. The egg hatches in about 72 hours, and the caudate first-instar larva begins to feed shortly thereafter. Approximately 2 days after hatching, during which period the parasite larva has molted twice, the host becomes obese and shiny yellowish in color; it soon turns purple and very quickly dies. After the death of the host, the last-instar larva cuts a hole in the aphid's venter and becomes partly or entirely exposed while preparing its cocoon. The normal white (nondiapause) cocoon is wider and longer than the aphid, and when completed is firmly attached to the plant surface with the still-inflated aphid skin resting on top of it. Quite often under natural conditions the aphid skin is blown off or otherwise dislodged from the *Praon* cocoon, which then looks like a white scale on the plant.

The parasite prepupal period lasts for 1 or 2 days, while the pupal period usually takes 4 to 5 days. The meconium is passed by the prepupa and consists of 10 to 12 small, shiny-black, oval-shaped pellets. As emergence nears, the developing pupa darkens. The parasite adult emerges by cutting a smooth circular hole through the cocoon with its mandibles, usually near the posterior end relative to the

[1] Hymenoptera: Braconidae and Eulophidae.

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aphid skin. This contrasts sharply with the irregular hole cut by hyperparasites, so that determination as to whether an empty cocoon was occupied by a primary or a hyperparasite can be made with some degree of accuracy. In *P. palitans* the cycle from egg to adult emergence takes from 11 to 13 days.

Adult longevity depends upon climatic conditions and availability of food and thus varies considerably. Under laboratory conditions, with honey as food, adult longevity ranged from 12 hours (at 100° F. and 30% R. H.) to 56 days (at 50° F. and 50% R. H.). At 28° F. and 34% R. H. the adults lived up to 10 days.

Praon palitans is a multivoltine species having possibly as many as 18 generations a year in the field between March and November. From November to March most individuals are in some state of facultative diapause. As photoperiod and temperature decrease in the fall, the percentage of individuals entering diapause increases rapidly. Experimentation has shown that diapause is induced through the aphid host, which in turn is conditioned by the physiological state of the alfalfa plant.[4] Alfalfa plant conditioning is apparently a result of decreased photoperiod and temperature. The diapause state is spent as a mature larva inside the so-called diapause cocoon, which is brown in color and much tougher than the normal white cocoon. This species also produces brown intermediate or "partial-diapause cocoons." A fairly accurate way to distinguish a diapause cocoon from a normal one is through an examination of its ventral side. In the diapause cocoon an extra layer of silk is laid down on the ventral surface, and the larva or pupa within becomes invisible, whereas in the normal and intermediate cocoons the larva or pupa is clearly visible from beneath. After certain exposures to moisture and low temperature, the diapause is broken and the larva pupates under warmer temperature conditions.[4]

At emergence the female wasp has from 35 to 65 fully developed eggs in each oviduct. The remaining one-third of the eggs are in various developmental stages in the ovaries. Oögenesis does not occur throughout the life of the female. However, due to the female's apparent need for rest periods more than one-half hour long after rapid oviposition in 10 to 15 hosts, it has been concluded that oviposition is probably extended over a longer period than commonly observed. This contrasts with certain species of *Trioxys* (*Trioxys*) and *Aphidius* (*Lysiphlebus*) that we have observed, which apparently are not restrained from laying their full complement of eggs in a very short time if hosts are available.

The sex ratio of *Praon palitans* is approximately 1:1.

Aphids of the genus *Therioaphis* are the only known hosts of this parasite, and *T.* (*T.*) *maculata* appears to be a preferred host. The parasite has also been reared from *T.* (*Myzocallidium*) *riehmi* (Börner), both in the field and in the laboratory at Riverside, California. According to Muesebeck (1956) it also attacks *T.* (*T.*) *trifolii* (Monell) in the eastern United States.

Praon palitans will attack and develop in any stage of *Therioaphis maculata*, including both alatae and apteri. A slight preference was shown in the laboratory for nymphs of the third instar, while least preference was shown for those of the first and second instars. The habit of ovipositing in fourth-instar or adult aphids appears to be a major reason for the rapid spread of *P. palitans* in California since the developing larvae inside the winged aphids are known to have been carried for at least 40 miles in this manner (van den Bosch *et al.* 1957).

The secondary hymenopterous parasites now known to attack *Praon palitans* in California are divided into two groups: (1) pupal parasites (Pteromalidae) and (2) larval parasites (Cynipidae and Encyrtidae). The pteromalids oviposit through the cocoon when *P. palitans* is in the late larval or prepupal stage. The cynipids and encyrtids oviposit in living aphids containing *Praon* eggs or larvae. The pteromalids, which so far have been reared from field collections of *P. palitans*, are *Pachyneuron siphonophorae* (Ashmead) and *Asaphes californicus* Girault. A single encyrtid species, *Aphidencyrtus aphidivorus* (Mayr), and at least four cynipids, all *Charips* species, have also been reared.

Trioxys utilis.—The female of this parasite is ready to oviposit immediately after emerging from the cocoon. With *T. utilis*, mating lasts about 30 to 45 seconds, and as soon as the act is completed the female is ready to oviposit. The *Trioxys* female deposits her egg internally, as does *Praon palitans*. Normally only 1 egg is deposited per insertion of the ovipositor, but occasionally 2 to 4 may be deposited. If this occurs, only 1 individual reaches maturity.

In attacking its host, *Trioxys utilis* exhibits a unique feature of grasping and holding the aphid by means of a three-pronged "clamp" formed by the two caudal abdominal spines and the ovipositor. This "clamping" is apparently an adaptation which has evolved to prevent saltatorial aphids such as *Therioaphis* spp. from leaping away from the parasite when attacked.

The egg of *Trioxys utilis* hatches about 48 hours after deposition, and in the ensuing 3 to 4 days the larva transforms through three instars. During the period of development of the last-stage larva, the host coloration changes progressively from greenish yellow to pink, and finally to dark purple, by which time the aphid has died. Approximately 5 days are required for the parasite to develop from egg to mature larva, and the prepupal period lasts about 1 day. The meconium, which is passed by the prepupa, is similar to that of *Praon palitans*.

The aphid mummy produced by *Trioxys utilis* differs from that of *Praon palitans* in that the aphid skin serves as the outer covering of the cocoon. Just prior to cocoon formation, the larva cuts a thin linear hole through the aphid venter and lightly fastens the aphid skin to the plant surface with silk. The cocoon may be brown or grayish white in color, with the former type predominating. Thus far, no biological differences have been detected between cocoons of the two color types. It seems possible that cocoon color may bear a relationship to temperature, white being more common in warmer weather (to reflect heat) and dark brown more common in cooler weather (to absorb heat).

Although there appears to be a certain amount of growth cessation (hibernation or quiescence) at cooler temperatures, no definite diapause individuals have yet been encountered either in the field or in the laboratory. The pupal period of *Trioxys utilis* ranges from 4 to 5 days,

[4] The diapause phenomenon in Aphidiine wasps will be discussed in a later publication.

and the total time required for parasite development from egg to adult emergence ranges from 9 to 11 days. The method of emergence of this adult parasite from the cocoon is almost identical with that of *Praon palitans* except that the exit hole is almost invariably near the dorsal posterior margin of the aphid skin.

Adult longevity is somewhat less than in *Praon palitans*, ranging from about 12 hours (at 100° F. and 30% R. H.) to 27 days (at 50° F. and 86% R. H.). At 28° F. and 34% R. H., adults lived up to 7 days. Field and laboratory data indicate that *Trioxys utilis* survives better at warmer temperatures than *P. palitans*.

Trioxys utilis is a multivoltine species having possibly as many as 24 generations a year. The female is nearly pro-ovigenic upon emergence, *i.e.*, her ovaries and oviducts are filled with fully developed eggs (from 200 to 300), except at the extreme base of each ovary, which contains a few undeveloped eggs in various stages of maturation that may or may not develop completely at a later date. This condition enables the female to oviposit immediately and quickly deposit her full complement of eggs if conditions are favorable. In this way *T. utilis* is similar to species of *Aphidius* and *Diaeretus* that we have observed in the laboratory.

The sex ratio of *Trioxys utilis* appears to be about 1:1.

The known hosts of *Trioxys utilis* all belong to the genus *Therioaphis*. The most important host in the Old World and in California seems to be *Therioaphis* (*T.*) *maculata*. It has been reported by Muesebeck (1956) as attacking *Therioaphis* (*T.*) *trifolii*. In 1957 van den Bosch recorded what he believed to be this species parasitizing *Therioaphis* (*Myzocallidium*) *riehmi* in Iran, and it has recently been reared from the same species on *Melilotus alba* Desr. at Riverside, California.

Although all stages of the host may be successfully parasitized by *Trioxys utilis*, there is a decided preference for the first three instars, and only rarely are adult aphids parasitized. In fact, considerable difficulty is experienced by this parasite in attempting to oviposit in larger aphids. Since most of the hosts attacked are young, and the parasite's larval developmental period is short, very few aphids reach full maturity before being killed and "mummified." Apparently for this reason only a small percentage of any population of *T. utilis* is carried from one area to another by the winged aphids. The much slower spread of *T. utilis*, as compared with *Praon palitans*, in California seems to be due to this factor.

Secondary parasites reared from *Trioxys utilis* in California are the same species as recorded for *Praon palitans*.

Aphelinus semiflavus.—A rather complete biology of this species has been presented by Hartley (1922), who used *Myzus persicae* (Sulz.) as its host. Certain salient features and new data are all that will be added at this time. However, it should be noted that the following information is based on the form of *A. semiflavus* that was introduced from the Middle East (mainly Israel), and on the basis of biological evidence there is reasonable doubt whether this Old-World strain is conspecific with the American form. The host used for all biological data recorded below was *Therioaphis maculata*.

With *A. semiflavus*, mating is not necessary for the production of progeny of both sexes. Laboratory tests have shown that male production from unmated females was greatly increased with higher temperatures, whereas higher percentages of females were produced as temperatures were lowered. For example, where the parasite was reared at temperatures of 65° to 72° F., about 2 to 5% male progeny were produced; but where reared at temperatures of 78° to 85° F., the proportion of males rose as high as 99%. *A. semiflavus* is apparently both uniparental and biparental, the sex ratio of the uniparental progeny, at least, appearing to be determined by environmental conditions (mainly temperature).

The *Aphelinus* female deposits an egg inside the host by a backward thrust of the ovipositor. The egg hatches in about 72 hours, and 3 to 4 days later the host turns black and dies. The parasite larva cuts a small hole in the venter of the dead aphid and attaches the skin to the plant surface with silk. The prepupal period lasts about 1 day; the pupal period ranges from 5 to 6 days. The total time required for *A. semiflavus* to develop from egg to adult is 11 to 13 days. Emergence of the parasite is invariably through a hole cut in the dorsal posterior margin of the host skin, the exit hole being somewhat rougher than that of either *Praon palitans* or *Trioxys utilis*.

Adult longevity in *A. semiflavus* is extremely variable. At 90° F. and 25% R. H., adults lived less than 12 hours; at 80° F. (25% R. H.), they lived less than 3 days; at 70° F. (60% R. H.) they lived about 28 days; at 60° F. (60% R. H.) and at 50° F. (86% R. H.) they lived up to 70 days; and at 28° F. (34% R. H.) up to 13 days. Further tests are being made to determine the effects of various humidities at the different temperature levels upon adult longevity in all three of the parasite species discussed in this report.

This multivoltine species, *A. semiflavus*, may have more than 20 generations a year. So far as is known it does not enter diapause, although it does have growth cessation periods in the colder months very similar to those known for *Trioxys utilis*. (It is also of interest to note that in 1940 Flanders reported that *Aphelinus mali* (Haldeman) entered into a diapause of undetermined length as a full-fed larva when subjected to temperatures above 110° F. at very low humidity.)

At emergence, the female *A. semiflavus* has only about 8 to 10 fully developed eggs in her oviducts. About twice this number are in various stages of development in the ovaries. However, Hartley (1922) has shown that a single female may deposit as many as 500 eggs during her lifetime, indicating that oögenesis is a protracted process. One of the most important biological characteristics of this species is its host-feeding habit. Quite frequently a female, after withdrawing her ovipositor from a host aphid, will turn around and with her mandibles widen the puncture and feed on the aphid's body fluids. As many as 50% of the stung aphids have been observed to be attacked in this manner, and the parasite is capable of consuming the entire liquid contents of a small aphid in a single feeding.

Although *A. semiflavus* will attack all growth stages of *Therioaphis maculata*, a decided preference is shown for first- and second-instar nymphs. Adult aphids are rarely attacked, and when they are stung the parasite frequently has great difficulty in removing her ovipositor.

The known hosts of the imported form of *A. semiflavus* in California are *Therioaphis* (*T.*) *maculata*, *T.* (*Myzo-*

callidium) *riehmi*, and *Myzus persicae*. There are, however, no less than 12 species in seven genera of aphids which are recorded as hosts for the native form of this parasite in North America (Muesebeck *et al.* 1951).

The secondary parasites reared from *A. semiflavus* are the same as those from *Praon palitans*.

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Aromatic Bait Traps as a Factor in Insect Spread[1]

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ABSTRACT

Aromatic bait traps, as used in insect survey, do not trap all of the specimens which they attract and therefore may be responsible for establishing new centers of infestation unless (1) each trap is located well outside the attractive range of that of any other trap, (2) each trap is located well away from the field hosts of the insect species concerned but still within attractive range of such hosts, (3) the amount of lure used is gauged to permit maximum attractive range without being repellent at trap source, and (4) areas immediately adjacent to trap locations are kept under continuous effective treatment, using non-attractive insecticides.

Various types of traps baited with aromatic lures are generally recognized and used as an effective tool in determining the presence and population density of an insect species in a given area. They are particularly useful when applied to the specific problem of detecting the possible presence of a *pest* species in a *new* area, to permit the immediate application of eradication measures to any incipient infestation found, and in delimiting the area that would thus be brought under treatment. No other form of survey could more effectively serve this purpose under the low population density involved.

Unfortunately, however, as is well known, *such traps do not trap all of the specimens which they attract.* If they did they would be the answer to eradication. Their injudicious use could, therefore, become a direct invitation to the establishment of new centers of infestation. Elimination of this obviously adverse trap characteristic seems very simple—merely assure that each trap is located well outside the attractive range of that of any other. It would thus serve its true detection purpose by operating as an independent unit, and attractive only to individuals already present within its range. Such practice would preclude any possibility of its becoming a link in a chain of traps progressively inviting spread and would have the added value of pin-pointing any existing infestation as lying within its attractive range. An exception to such distribution would be trap concentration designed to pull all existing infestation toward a common center as an aid to eradication. However, it would again become applicable when locating traps in relation to the periphery of the area placed under eradicative treatment, by assuring that they were well beyond their attractive range.

While, as previously stated, the method of elimination of this adverse trap characteristic is simple, its accomplishment is a little more difficult and dependent entirely on a knowledge of the attractive range of the lure used. Specific determination of such range under field conditions is practically impossible, primarily due to the unpredictable variation in the direction and velocity of prevailing winds, and with respect to the factors affecting the rate of volatilization of the lure. Where fruit flies have been concerned workers have found that the distance may range from 100 yards or less in the case of males of the melon fly and the Mediterranean fruit fly, to one-half mile in the presence of an 8-mile wind, in the case of males of the Oriental fruit fly. In most cases it becomes necessary to accept a reasonable estimate based on accumulated experience. To compensate for any unfavorable error and allow for any unusual increase in wind velocity, it would seem reasonable and much safer to double that estimate when locating traps. Fortunately, when wind velocities become excessive insects seem reluctant to voluntarily take to the air, regardless of any outside attraction.

The amount of lure used at trap source can also be a

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major factor contributing to the adverse effects of trap use. It is firmly believed that insects are attracted more by an elusive scent than by one of high potency. If this is true the amount used should be sufficient to give maximum attractive range and, at the same time, not so strong as to be repellent at the trap. Otherwise, it would materially increase the number of individuals attracted but not trapped and to the same degree increase the possibility of establishing infestation within the trap area, as well as increase the numbers that might be attracted to other trap areas. Failure to observe these limitations in the lure may be the basic reason for trap failure to pick up all of the individuals which it attracts—many being repelled or diverted as they approach the progressively increasing odor source.

Some authorities on insect detection survey contend that if the traps used are too widely separated they cannot be depended on in areas of low population density. Seeming to confirm that thinking, workers associated with the recent Mediterranean fruit fly eradication project in Florida reported that traps located 800 to 1000 feet apart over a wide area, or at the rate of 20 to 30 per square mile, in the presence of extremely light infestation, failed to detect fly populations below that density represented by the reproduction of two generations. Tending to refute that conclusion, trapping by California authorities, using traps and lures similiar to those used in Florida, but much more widely separated than the limits quoted, picked up a single specimen of melon fly in an area under survey which subsequent intensive trapping has failed to duplicate. During following years no evidence of established infestation has been uncovered.

In another instance, survey for Oriental fruit moth in the great central valley in California in the early '40s recorded single moths being trapped at each of several locations some of which were separated by as much as 100 miles which subsequent intensive trapping and visual host inspection never later duplicated. On three widely separated occasions over a period of years, single specimens of Japanese beetle were taken during token trapping conducted adjacent to the approach area to major airports in California—twice at Los Angeles and once at Fairfield in the north-central part of the State—which have never been duplicated by subsequent intensive trapping in the respective areas, nor has established infestation ever been indicated. It is hard to believe with respect to the several instances cited, that the only existing specimen, in each case, was trapped. However, they do seem to confirm that such traps, even though widely spaced, are more highly effective in the presence of obviously extremely low populations, than others suspect.

Contributing further to the adverse characteristics of lure-baited traps is their tendency to bring the two sexes together where such meeting would otherwise be highly problematical under the low population densities represented by incipient infestations. This would tend to assure establishment of infestation in the trap area by mated females which had not been trapped.

To correct another adverse situation traps should be so located as to attract specimens away from rather than to their hosts as the latter may be the more attractive when placed in local competition with the lure. Infestation might result from the activity of individuals attracted to the vicinity by the longer range of the trap.

Even when each trap area is arranged so as to be beyond the influence of any other, the activity of individuals which they attract but do not trap, could, and should, be discouraged by keeping all hosts and soil within the immediate vicinity of each trap, under continuous treatment, using effective but non-attractive insecticides. This thought is not concurred in by some authorities who feel that the suggested treatment would hold populations below levels that would be recorded by any trap. This would seem to be refuted by the instances previously cited.

In summary, aromatic bait traps, as used in insect detection surveys, can actually be a material factor in the further spread of the species against which they are directed, through the activity of individuals which are attracted but not trapped, unless (1) each trap is located well outside the attractive range of that of any other trap, (2) each trap is located away from any field hosts of the insect species concerned, but within attractive range, (3) the amount of lure used is gauged to permit maximum attractive range without being repellent at source, and (4) areas immediately adjacent to trap locations are kept under continuous effective treatment, using non-attractive insecticides.

NOTICE TO MEMBERS

A new periodical, *Miscellaneous Publications of the Entomological Society of America* will be initiated by the Society in 1959. It will be devoted to entomological articles of more than 20 printed pages and of a type and length less than that suitable for the Thomas Say Foundation series.

If you have questions or a suitable manuscript please write the Executive Secretary, Entomological Society of America, 1530 P Street, N.W., Washington 5, D. C.

Biology of *Collops vittatus* (Say) in Arizona, and Feeding Habits of Seven Predators of the Spotted Alfalfa Aphid[1]

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Abstract

Studies on the biology of *Collops vittatus* (Say) were undertaken in the laboratory and field at Tempe, Ariz., from June 15 to September 15, 1957. On an average 78 days were required for development from egg to adult. Additional data on longevity and feeding capacity of *C. vittatus* in relation to temperature are given. Observations on the feeding habits of seven predators of the spotted alfalfa aphid (*Therioaphis maculata* (Buckton)) indicated that the average number of aphids destroyed per adult per day by *Hippodamia convergens* G.-M. was 97, *Collops vittatus* (Say) 35, *Zelus renardii* Kolenati 34, *Geocoris punctipes sonoraensis* Van Duzee 29, *Sinea confusa* Caudell 24, *Nabis ferus* (L.) 22, and *Orius tristicolor* (White) 5. The effect of sex and different feeding periods on the feeding capacity of the various predators is also given.

Since the appearance of the spotted alfalfa aphid (*Therioaphis maculata* (Buckton)) in the United States, several workers have reported the abundance of certain insect predators and their ability to destroy this aphid (Dickson *et al.* 1955, Smith & Hagen 1956, Bieberdorf & Bryan 1956). Observations in Arizona have clearly indicated an increase in predator activity, notably of *Collops vittatus* (Say), in infested alfalfa fields. Little was known of the life history and habits of this predator, particularly the larval stage, until Walker (1957) reported his observations in Texas. Walker's results were similar in some respects to those being reported here. However, differences observed on certain aspects of the life history in Arizona appeared great enough to warrant presentation of the entire study.

The biology and habits of *Collops* and its effectiveness as a predator on the spotted alfalfa aphid were studied in the laboratory at Tempe, Ariz., during 1957. Data were also obtained on the comparative effectiveness of several other predators, including the convergent lady beetle (*Hippodamia convergens* G.-M.), two reduviids (*Zelus renardii* Kolenati and *Sinea confusa* Caudell), the big-eyed bug (*Geocoris punctipes sonoraensis* Van Duzee), a damsel bug (*Nabis ferus* (L.)), and a minute pirate bug (*Orius tristicolor* (White)). According to Dickson *et al.* (1955), *Orius*, *Geocoris*, and *Nabis* were rarely seen attacking the spotted alfalfa aphid in the field. Field studies in California reported by Smith & Hagen (1956) indicated that the *Hippodamia* adult ate about 40 to 50 aphids a day, *Nabis* and *Geocoris* four aphids a day, and *Orius* rarely took aphids. The last two predators fed mostly on spider mites. All the predators were fed spotted alfalfa aphids exclusively and no attempt was made to determine their preference for various prey. Nonetheless, some indication of the potential number of aphids that might be destroyed in the field could be obtained.

Life History and Habits.—Life-history studies on *Collops vittatus* were conducted in the laboratory and field from June 15 to September 15. Large quantities of beetles were collected in fields and brought to the laboratory, where individuals of each sex were paired and confined in plastic dishes for mating. Daily observations were taken on copulation, pre-oviposition, and oviposition. Following egg deposition, the adults were transferred to clean dishes for further study. After the eggs hatched, the incubation period and number of larvae were recorded. One hundred first-instar larvae were placed in groups of 10 in plastic dishes and observed daily. After the larvae reached the prepupal stage, they were placed individually in small dishes containing soil for pupation. The adults and larvae were fed a daily diet of spotted alfalfa aphids during the entire study. A hygrothermograph was used to record the temperature and humidity.

Data on the duration of the various stages in the life of *Collops* are summarized in table 1.

The sex of the adults is readily distinguished by the male having a greatly enlarged third antennal segment. The beetles are brightly colored and very active. Prior to copulation the male persistently followed the female, frequently touched her abdomen with his antennae, and finally assumed a posterodorsal position on her body. During coition the female energetically undulated her body laterally for about an hour in an apparent attempt to break the male's hold. Gradually her activity decreased to a motionless, almost stuporlike condition which lasted for an hour; then the beetles separated. Many of the beetles mated more than once and some of them four or five times.

The female usually laid a single cluster of 25–40 eggs after each copulation period, but sometimes two clusters were laid 1 or 2 days apart. Most of the eggs were laid underneath a layer of filter paper that had been placed in the dish, although some were on the side of the dish or on aphid-infested alfalfa leaves placed in the dish. The eggs were held fast by a colorless sticky material exuded by the female. Many females laid more than one cluster, and

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[2] In cooperation with the Arizona Agricultural Experiment Station.

Table 1.—Duration of the stages in the life of *Collops vittatus*, June 15 to September 1, 1957.

Stage	Number of Records	Period[a]	
		Range	Average
Copulation	20	2– 4	2.6
Pre-oviposition	20	5–13	8.0
Oviposition	100	1– 4	2.5
Incubation	1700	3– 8	5.5
First instar	10	5–15	8.0
Second instar	10	4– 8	5.8
Third instar	10	8–16	13.0
Fourth instar	10	11–19	12.2
Fifth instar	10	12–16	14.3
Prepupa	4	2– 5	3.2
Pupa	4	6–12	8.5

[a] Hours for copulation and oviposition; days for other stages.

Table 2.—Measurements (in millimeters) of eggs and larvae of *Collops vittatus*.

Stage	Number of Records	Body Length		Head Capsule			
				Length		Width	
		Range	Average	Range	Average	Range	Average
Egg	22	0.90–1.01	0.95	— —	—	— —	—
First instar	6	1.57–1.71	1.65	0.200–0.214	0.207	0.214–0.228	0.218
Second instar	11	2.20–3.30	2.90	0.257–0.300	.283	0.271–0.300	.289
Third instar	12	3.70–4.60	4.23	0.328–0.385	.355	0.342–0.428	.396
Fourth instar	13	6.10–7.50	6.62	0.600–0.830	.711	0.630–0.830	.733
Fifth instar	13	8.50–9.20	8.85	0.730–0.930	.815	0.760–0.930	.828

some of them as many as five clusters. The greatest number of eggs in a cluster was 110, but the average number per female by 66 females was 40.5. Walker (1957) reported an average of 580 eggs per female. This may have been due to better diet, since he fed the beetles an assortment of eggs, pupae, and freshly killed adult insects.

The egg was about 1 mm. long, cylindrical, tapered at both ends, and bright orange when freshly laid. Prior to hatching the color turned from bright to pale yellow and finally whitish. A dark eyespot appeared on the anterior end about 2 days before hatching. The chorion became wrinkled and dry just before the weak, pale pinkish larva emerged. Egg measurements are presented in table 2.

In the early larval instars the head and urogomphi were heavily sclerotized and the rest of the body was soft and sparsely clothed with fine, hairlike setae. In later instars the body was sclerotized throughout. Larvae were campodeiform with the body orthosomatic and compressed dorsally. The head was slightly narrower than the thorax. Thoracic segments were equisomatic and twice as long as abdominal segments. The abdomen was nine-segmented, the last segment bearing a pair of immovable urogomphi with apices curved dorsad. Color was pale pinkish in the first instar to deep orange red in the last instar. Larvae were cannibalistic, the larger larvae feeding on smaller ones. Food preference was small live aphids and freshly killed individuals. Measurements of the larvae are given in table 2.

The prepupal stage started after the abdomen of fifth-instar larva became distended and the entire body immobile. The pupa was exarate, and the last larval skin was usually attached loosely to the posterior part of the abdomen. Pupation occurred just under the soil surface.

A Berlese funnel was used to collect soil, debris, litter, and plants to determine the habitat of the larvae. Soil samples taken to a depth of 6 inches revealed no larvae. Many samples of debris and humus revealed hundreds of larvae of all instars except the first. No larvae were found in or on alfalfa plants. The larvae apparently live in the humus, feeding mainly on small living insects and dead insects of larger size. They may be considered semipredaceous.

Adult numbers and activities were observed in alfalfa fields by sweep-net collections and examination of plants and the soil surface upon which the beetles lived. Under confinement the adults were cannabilistic, but otherwise very efficient predators in the field, where they sometimes outnumbered the convergent lady beetles. They were found mostly on foliage feeding on spotted alfalfa aphids and other insects. Occasionally they were on the ground where they apparently deposited their eggs under bits of dead plant matter and other debris. A few eggs were found on alfalfa leaves. The beetles were present in the fields throughout the year, but most abundantly from March through October with a population peak in August (Barnes 1958).

ADULT LONGEVITY AND FEEDING CAPACITY.—Longevity studies of *Collops vittatus* were based on the length of life of each beetle held in captivity and subjected daily to a constant diet of a given number of spotted alfalfa aphids. The experiment covered the period from June 21 to September 4. Beetles were collected in the field, brought to the laboratory, and placed individually in petri dishes together with aphid-infested alfalfa leaves. Moist filter paper was placed in the bottom of each dish to provide moisture for the beetles and to keep the leaves from drying out. Each composite number of aphids was fed to five adults of each sex. Beetles were fed every morning by collecting infested leaves and introducing the desired number of aphids into each dish. The dishes were observed daily for dead beetles until all the beetles had died.

As shown in table 3, beetles that were fed 20 aphids per day lived the longest. It seems that those fed 40 aphids per day should have lived longer, but by chance older beetles may have been selected during the random collection and placement in the petri dishes. On the other hand, some factor associated with large numbers of aphids may be detrimental to the beetles under confinement. The males lived longer than females except under starved conditions. Data on males that were fed 10 aphids per day were not considered valid, as three of them escaped before the tests were completed. Males feeding on 20 aphids per day lived 10 times as long as males that were starved, but females on the same diet lived only four times as long. The maximum longevity for a single adult was 77 days for a male fed 40 aphids and 75 days for a female fed 20 aphids per day.

Table 3.—Longevity (in days) of *Collops vittatus* after feeding daily on a constant diet of given numbers of spotted alfalfa aphids. June 21–September 4, 1957. Tempe, Ariz.

Number of Aphids Fed Daily	Males	Females
40	30.6	26.4
20	44.8	40.2
10	13.7	38.4
5	14.6	13.7
0	4.4	9.2

Table 4.—Number of spotted alfalfa aphids consumed by *Collops vittatus* at different periods of each day for 45 days.

PERIOD	MALES	FEMALES
5 P.M.–9 A.M.	2199	2204
9–11 A.M.	1522	1465
11 A.M.–1 P.M.	1184	1403
1–3 P.M.	1085	1463
3–5 P.M.	962	1437
Total	6952	7972
Average per day	22.7	27.3
Maximum for single beetle	1079	1077

Feeding capacity of *Collops vittatus* was based on the total and daily average numbers of spotted alfalfa aphids consumed after five feeding periods per day for 45 days. The results are summarized in table 4. Ten adults of each sex were used at the beginning of the experiment, but by the end of the tests 80% of the females and 40% of the males had died. Aphid consumption by the females was about the same during all feeding periods, except at night when they had many more hours to feed. The number of aphids eaten by the males decreased during the day. Both sexes consumed about the same number of aphids during the night, but the females ate more during the late morning and afternoon periods.

TEMPERATURE RELATIONSHIPS.—Investigations on the relation of temperature to feeding capacity of *Collops vittatus* covered the period from June 25 to August 8. Beetles were collected in the field and placed individually in 1-×5-inch glass tubes stoppered at both ends. Ten adults of each sex were fed daily at 9 and 11 a.m. and 1, 3, and 5 p.m. Infested leaves having a known number of aphids were introduced into each tube at feeding time. Before a new leaf was introduced, the aphids that remained from the previous feeding were counted to determine the number eaten or destroyed for that period.

Relation of mean temperature to the number of spotted alfalfa aphids consumed over a 45-day period is shown in table 5. For the most part, aphid consumption by both sexes increased with the temperature.

COMPARATIVE FEEDING HABITS OF SEVEN PREDATORS.—This study was undertaken from July 16 to 23. Ten adults of seven predators were collected in the field and placed individually in 1-×5-inch glass tubes stoppered at both ends with cheesecloth. Each predator was represented by 5 males and 5 females, except *Orius tristicolor*, which was not separated as to sex. The adults were fed spotted alfalfa aphids each day at 9 a.m. and 1 and 4 p.m. The approximate number of aphids eaten was determined first by exploratory feeding tests and a slight excess of aphids was supplied to insure maximum feeding for each period. The method of feeding was the same as in the temperature-relationship tests.

The number of spotted alfalfa aphids eaten or destroyed over an 8-day period by adults of seven predators is summarized in table 6. By the end of the study the mortalities of the predators were as follows: *Hippodamia* 30, *Collops* 20, *Zelus* 10, *Geocoris* 10, *Sinea* none, *Nabis* 30, and *Orius* 50%.

The beetles destroyed their prey by consuming the entire bodies and thus were much more thorough than the hemipterous predators. The convergent lady beetle

Table 5.—Relation of mean temperature to number of spotted alfalfa aphids consumed by *Collops vittatus*, June 25 to August 8, 1957.

TEMPERATURE (° F.)	AVERAGE NUMBER OF APHIDS CONSUMED PER PREDATOR	
	Males	Females
81	12.0	17.5
82	18.6	25.1
83	23.5	20.6
84	22.2	19.0
85	16.6	15.8
86	18.5	15.0
87	23.7	23.8
88	22.1	28.0
89	22.2	28.0
90	29.3	33.6
91	25.7	34.8
92	28.6	33.5

was the most efficient and is by far the most important predator of the spotted alfalfa aphid in Arizona. In the laboratory it ate twice as many aphids as it did under field conditions in California. It had an extremely voracious appetite and consumed an entire aphid in a few seconds.

Collops vittatus has become an increasingly important predator of the spotted alfalfa aphid and ranks second to the convergent lady beetle with regard to populations in the field. This was the only predator in which the females ate nearly twice as many aphids as did the males. It consumed aphids less efficiently and more slowly than the convergent lady beetle did.

The hemipterous insects killed aphids by sucking out their body fluids. Although haphazard in their feeding habits, most of them were efficient predators. The most remarkable predator was the reduviid *Zelus*, which consumed nearly as many aphids as did *Collops*. It sucked out their body fluids, then cast the dried bodies to one side. Most of the smaller aphids were simply killed by the predator's mouth parts and discarded, since they offered little body fluid as food. Populations of this predator were generally smaller than those of other predators. *Geocoris*, well known for its predatory habits on the aphid and other arthropods, ranked third in respect to populations in the field. However, in the laboratory it did not destroy as many aphids and was not as efficient as *Collops*. The predator was very active and often ran around infested leaves killing more aphids with its mouth parts than by actual feeding. The reduviid *Sinea* was slow moving and killed its prey by inserting its mouth parts and sucking out the body fluids. Larger aphids were preferred as food, although it attacked smaller ones. *Nabis* was not an effective predator but it sucked out all the body fluids of each aphid, large or small. *Orius* was the most inefficient predator studied. Mostly small nymphs were attacked, and they were rarely consumed. As indicated by Smith & Hagen (1956), *Orius* is more important as a predator on eggs and adults of spider mites.

The effect of sex and time of day on the feeding capacity of these predators is shown in table 7. Considerable variation is evident between sexes of the same predator and between adults of different predators. In spite of the longer nighttime feeding period, aphids consumed or

Table 6.—Number of spotted alfalfa aphids eaten or destroyed by adults of seven predators. July 16 to July 23, 1957.

Predator and Sex	July 16	17	18	19	20	21	22	23	Average per Day	Total
Hippodamia										
Female	97.2	87.8	110.2	93.5	124.5	110.3	101.2	74.5	96.6	
Male	101.8	112.6	78.7	104.5	126.3	107.8	98.3	78.6	96.9	6195
Collops										
Female	47.0	44.8	45.2	33.1	58.6	41.6	29.3	52.0	42.6	
Male	39.2	28.0	27.6	35.2	40.6	30.5	18.2	20.5	28.5	2633
Zelus										
Female	24.8	21.0	24.8	42.6	52.2	60.6	27.7	34.3	33.1	
Male	26.2	33.2	27.8	57.6	58.8	47.5	23.6	39.6	35.6	2649
Geocoris										
Female	25.4	25.6	28.2	43.2	47.4	34.2	21.8	26.5	30.0	
Male	27.4	26.2	27.0	31.7	45.4	39.3	12.7	18.7	28.1	2103
Sinea										
Female	18.6	18.8	18.6	29.4	55.6	32.0	8.8	23.7	24.1	
Male	18.6	20.0	30.6	30.2	47.0	28.7	7.8	19.7	24.1	1927
Nabis										
Female	23.2	22.7	21.7	23.0	28.0	22.1	17.6	17.5	22.6	
Male	14.4	22.5	20.7	24.0	44.2	31.2	22.5	6.3	22.1	1390
Orius	6.3	5.6	4.3	7.5	7.0	6.5	2.6	2.8	5.1	293

destroyed by both sexes of all predators were greatest during the afternoon period.

Females of *Hippodamia* ate a few more aphids during the night than the males, but both sexes consumed about the same number during the morning and afternoon periods. During all periods of the day females of *Collops* consumed more aphids than males but males of *Zelus* destroyed more aphids than females. Aphids destroyed by each sex of *Geocoris* and *Sinea* were approximately the same for all periods, except *Sinea* males killed a few more than females did during the night. Females of *Nabis* destroyed more aphids than the males did during the night, but the males destroyed more during the afternoon. Aphids destroyed by both sexes of *Nabis* were approximately the same during the morning. *Orius* killed most of its prey during the afternoon.

Since the greatest number of aphids was consumed or destroyed by the various predators during the afternoon period, it appeared that such activity was strongly governed by higher temperatures during this time. Such phenomena occurred in relation to temperature and feeding capacity of *Collops vittatus* mentioned previously.

Table 7.—Number of spotted alfalfa aphids consumed or destroyed during different periods of day by each sex of seven predators. July 16 to 23, 1957.

Predator	Night		Morning		Afternoon	
	Females	Males	Females	Males	Females	Males
Hippodamia	35.3	33.5	27.8	27.6	41.8	42.1
Collops	15.2	9.0	11.6	7.2	18.9	15.5
Zelus	9.7	13.6	8.3	9.4	13.7	15.1
Geocoris	10.6	9.2	6.9	6.3	14.0	13.9
Sinea	6.8	8.1	6.1	6.5	11.7	11.0
Nabis	7.3	4.8	5.0	5.4	12.0	14.5
Orius	0.7		1.3		3.5	

Discussion.—Data presented herein on the life history of *Collops vittatus* differ somewhat from those reported by Walker (1957). Most notable is the tremendous disparity in egg deposition. Also, measurements of the eggs and larvae indicate that the Arizona specimens were larger. Some of the descriptions and measurements appeared to fit closely *Collops balteatus* LeConte of Walker's work. One series of specimens sent to the U. S. National Museum were determined as *Collops* sp., probably *vittatus* (Say) and a similar series as *Collops vittatus* (Say). This suggests that either geographical variation or a different species is involved. A certain amount of clarification on the problem seems necessary, mainly from the taxonomic viewpoint.

Although most of the predators consumed or destroyed more aphids in the laboratory than they did under field conditions in California, additional information is needed on other factors, such as host preference and relative abundance of other prey, before a valid appraisal of each predator can be ascertained. Data presented here on the feeding capacity of each predator with respect to the spotted alfalfa aphid will be useful in studying feeding habits in the field.

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The Biotic Effect of Insecticides on Populations of Aphids and Mites of Pecans.[1]

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ABSTRACT

Research was performed to determine the factors which contribute to increases in aphid and mite populations on pecans following the use of certain insecticides.

Aphid and mite predators had little inhibiting influence, since they were present in equal numbers on both sprayed and unsprayed trees.

Tests were performed to determine whether DDT increased the reproductive rate of the black-margined aphid, *Monellia costalis* (Fitch), and the black pecan aphid, *Melanocallis caryaefoliae* (Davis). No aphids survived on treated leaves until the residue diminished. After this period, populations were comparable in sprayed and unsprayed leaves, indicating that stimulation by DDT was not a significant factor.

Mechanical factors, such as leaf pubescence and surface residues, appeared to be significant influences on the development of aphid and mite (*Tetranychus hicoriae* McGregor) infestations following the use of certain insecticides on pecan trees.

Considerable discussion has arisen concerning the factors contributing to aphid and mite population increases on pecans and many other crops following the application of certain insecticides. Steiner *et al.* (1944) observed large populations of a spider mite, *Tetranychus* sp., at harvest time on the fruit and under the bark of apple trees sprayed with DDT. Few mites were found on unsprayed trees. English & Tinker (1954) conducted a two-year study on the effect of DDT on the two-spotted spider mite, *Tetranychus telarius* (L), and the aphid, *Tuberculatus ulmifolii* Monell, on elms. These authors observed that the application of a DDT spray on elm trees resulted in increased mite populations which were five times larger than those on unsprayed elms. Aphid populations also increased on the DDT-sprayed trees.

In studies primarily concerned with the control of the pecan nut casebearer, *Acrobasis caryae* Grote, Pierce (1953) noted that outbreaks of the mite, *Tetranychus hicoriae* McGregor, followed the application of DDT. King & Rosberg (1956) tested a number of insecticides to determine their effects on mite populations. Mite populations increased following the application of each insecticide used except DDT plus sulfur.

Davis (1952) conducted an extensive study of increases in mite populations following the application of DDT on banana squash. Results of a series of experiments indicated that mites became irritated following contact with DDT, and migrated rapidly to other leaves on the host. During the first few days following the application of DDT, fewer eggs were deposited by treated mites. Eleven days after the mites were sprayed with DDT, normal egg production was resumed. The sprayed mites, under less crowded conditions produced offspring for a longer period. Egg production per female mite was about three times greater where ample space and better feeding conditions were available. Davis concluded that some of the mite predators, important in limiting mite populations, are killed by DDT. He attributed mite population increases to the high mortality rate of predators following the application of DDT and the higher reproductive potential of the mites resulting from dispersion on the host.

Increased populations of mites caused by dispersion on pecan trees could be a significant factor if mite populations were large enough to produce crowded conditions on the host plant at the time sprays are applied. However, a period of several weeks frequently elapses between the final application to pecans and the development of severe mite infestations. This study (Warren 1958) was undertaken to determine the factors which contribute to the development of large populations of aphids and mites on pecan trees sprayed with DDT.

EFFECTS OF DDT ON APHIDS AND MITES.—Before determining the reason for increased populations of aphids and mites on pecan trees following the application of DDT, it was necessary to determine whether populations of these arthropods increased following the use of DDT in this area. Four pecan trees were selected, all of which were subject to similar environmental conditions. Two were sprayed with DDT and two served as checks. A pre-spray record of aphid and mite populations was made on May 2, by selecting 10 leaves at random from each tree. Pecan leaves are composed of a rachis which usually bears nine or 11 leaflets. No aphids or mites were recorded at this time when the nuts were approximately $\frac{1}{4}$ of an inch long. Small populations of black-margined aphids, *Monellia costalis* (Fitch), were present on May 14 when a second pre-spray examination was made.

On May 21, two of the trees were sprayed with 1.5 pounds of DDT per 100 gallons of water as an emulsion spray, at a pressure of 400 p.s.i. On May 23 a 75% decrease in the aphid infestations on the two sprayed trees was recorded. Populations on the two unsprayed trees increased at a regular rate. Populations on the sprayed trees began to increase on May 27. Peak populations of aphids were reached on June 11. Sprayed trees were twice as heavily infested as unsprayed trees. Aphid populations then began to decline until comparable levels were attained on both sprayed and unsprayed trees.

A second spray application was made on June 19. Few aphids and mites, *Tetranychus hicoriae* McGregor, were present on the sprayed trees for 3 weeks following the application. At the end of this period on July 16, mite populations began to increase on the sprayed trees. Black-margined aphids failed to repopulate the test trees.

A severe mite infestation occurred from July 16 until August 27 on the sprayed trees. The population fluctuated during that period, peak numbers being attained on July 23. Throughout this test period, large populations of aphids and mites did not develop on the check trees, while

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[2] Former Research Assistant, and Associate Professor, respectively, Department of Entomology.

infestations on sprayed trees were relatively high, demonstrating that aphids and mites increased following the application of DDT.

EFFECT OF PREDATORS ON APHID AND MITE POPULATIONS.—To determine the effect of predators on aphid and mite populations on pecans, an experiment was conducted on trees that had been sprayed with insecticides on May 21, to control the pecan nut casebearer. The experiment was conducted to determine the correlation between the number of aphid and mite predators with aphid and mite populations on sprayed and unsprayed trees and was replicated four times using six insecticides and a check. Counts of aphids and mites and their arthropod predators were made at weekly intervals on 10 leaves selected at random from each tree.

A few ladybird beetles were observed during the season. Populations of other predators were negligible. Dorman (1934) considered lacewingflies, *Chrysopa* spp., the most important predators of black-margined aphids. They also feed on black pecan aphids, *Melanocallis caryaefoliae* (Davis), and mites. Lacewing larvae were rarely observed. As a result, they had little influence on aphid and mite populations. Records were kept, however, of the number of lacewing eggs observed each week. Many eggs hatched, but the larvae could not be located.

The insecticidal treatments apparently afforded no resistance to the development of lacewing populations on the basis of the number of eggs present on treated and untreated trees. The smallest number of eggs was observed on untreated trees and on those treated with endrin (table 1).

Plots treated with endrin and the untreated check plots also had the smallest number of aphids, with the exception of trees treated with malathion (table 2).

The smallest number of mites occurred on the untreated check plots and on trees treated with malathion (table 3).

These observations indicate that aphid and mite predators had little influence in maintaining aphid and mite populations at noninjurious levels. The endrin-treated trees were infested with a large number of mites and a small number of lacewing eggs, but this was the only treatment yielding such an indication. Trees treated with DDT or Diazinon had the greatest number of lacewing eggs, the largest number of mites, and a comparatively large number of aphids. These observations indicate that insecticides have little inhibiting influence on aphid and mite predators. Since the aphid and mite predators had little or no effect on the populations of aphids and mites following the application of certain insecticides, experiments were conducted to determine (1) whether there was a physiological effect of DDT on pecan trees which might cause an increase in aphid and mite populations, and (2) whether there was a direct physiological effect of DDT on aphids and mites.

Table 1.—Lacewing egg populations following the application of insecticides on May 21 for control of the pecan nut casebearer.

Treatment	Dates (1958)									
	6/11	6/18	6/25	7/2	7/9	7/16	7/23	7/30	8/6	Total
DDT	8	4	4	1	1	0	0	1	1	21
Malathion	5	5	3	1	0	0	1	0	0	15
Toxaphene	5	6	3	1	0	0	0	0	0	15
DDT plus sulfur	15	3	0	0	0	0	0	0	0	18
Endrin	0	1	2	0	0	0	0	0	0	3
Diazinon	0	0	12	8	1	0	0	0	0	21
Check	0	5	0	1	0	0	0	0	0	6

Table 2.—Black-margined aphid populations following the application of insecticides on May 21 for control of the pecan nut casebearer.

Treatment	Number of Aphids per 10 Leaves									
	6/11	6/18	6/25	7/2	7/9	7/16	7/23	7/30	8/6	Total
DDT	8	17	13	15	2	8	4	3	0	70
Malathion	2	2	9	6	3	0	0	1	2	25
Toxaphene	9	7	6	11	0	4	2	4	1	44
DDT plus sulfur	4	21	4	10	0	6	2	6	3	56
Endrin	1	6	8	2	3	0	9	6	2	37
Diazinon	1	13	0	1	10	6	2	4	2	39
Check	1	13	6	2	0	5	2	0	0	25

PHYSIOLOGICAL EFFECT OF DDT ON PECAN TREES.—In the first experiment, polyethylene bags were used to confine black-margined aphids to DDT-sprayed leaves. Black-margined aphids were also confined to unsprayed leaves to serve as checks. Four vigorous leaves, consisting of approximately 40 leaflets, were selected on an unsprayed pecan tree. The leaves were sprayed, using an atomizer, with the equivalent of 1.5 pounds technical DDT per 100 gallons of water. On June 12, after the spray had dried, 10 aphids were confined on the sprayed leaves in polyethylene bags which had been punctured previously to permit circulation of air. The bags were placed over the leaves and were tied securely around the leaf bases to prevent the escape of aphids. Four other leaves were selected from the same side of the tree to serve as checks. Ten aphids were confined to these leaves on the same date. Aphids confined on the sprayed leaves were repelled and all killed by the DDT within 1 hour. The aphids confined on the unsprayed leaves survived.

Aphid populations within the bags were checked at weekly intervals. All of the aphids on the unsprayed leaves were removed following each examination. Ten winged females were again confined to the same leaves in polyethylene bags. Treated leaves were reinfested each week with aphids collected from the test tree until they survived within the bags. When aphids began to survive on the treated leaves, 10 winged females within the bags were selected and replaced following each examination. The remainder of the aphids were brushed off the leaves. Aphids confined to the DDT-sprayed leaves failed to survive until the end of the fifth week. From that time until the end of the experiment, the weekly examinations revealed comparable populations of aphids on both sprayed and unsprayed leaves.

A second experiment, using paired trees, was also performed using the black-margined aphid. The treated tree was sprayed with DDT on May 22, the other was unsprayed. On June 19, polyethylene bags were placed over the leaves of both trees and infested with aphids in the manner described above. By July 17, the sprayed tree supported a relatively large population of aphids, the unsprayed tree had very few. Aphid populations remained comparable on the sprayed and the unsprayed leaves within the polyethylene bags. The same procedures were used to test the effect of insecticides on the black pecan aphid and *T. hicoriae*. Comparable results were obtained. Under the conditions of these experiments, aphids

Table 3.—*T. hicoriae* populations following the application of insecticides on May 21 for control of the pecan nut casebearer.

Treatment	Dates (1958)									Total
	6/11	6/18	6/25	7/2	7/9	7/16	7/23	7/30	8/6	
DDT	1	100+	100+	100+	100+	100+	26	8	14	549+
Malathion	1	3	4	13	19	29	24	7	4	103
Toxaphene	1	13	13	78	78	37	26	14	25	286
DDT plus sulfur	0	6	11	70	59	28	100+	30	16	320+
Endrin	1	100+	31	90	86	33	74	24	19	458+
Diazinon	0	1	5	22	21	90	100+	32	38	309+
Check	0	11	8	13	76	25	9	2	25	173

and mites were not stimulated into a greater reproductive potential by a physiological response of the tree to DDT.

PHYSIOLOGICAL EFFECT OF DDT ON APHIDS.—To determine whether there was a direct physiological effect of DDT on aphids, black-margined aphids were sprayed with DDT, then confined to unsprayed leaves by the use of polyethylene bags. The concentration of the insecticide used was equivalent to 1.5 pounds of technical DDT per 100 gallons of water. This experiment was replicated 10 times using 10 aphids in each bag. No aphids survived the treatment.

The possibility of aphids being stimulated by DDT to produce a normally greater number of offspring is very remote. Every instance in which aphids were subjected to DDT, whether the means of subjection was by direct spraying of aphids or by placing them on a freshly sprayed surface, dry or wet with the insecticide, mortality was 100%.

Black-margined aphids, black pecan aphids, and two species of mites, *T. hicoriae* and *Oligonychus viridis* (Banks) were subjected to applications of DDT spray under field conditions. The first application resulted in 100% mortality of all species. After a period of approximately 1 week, *T. hicoriae* was present in numbers comparable to those prior to spraying. Probably, the eggs present on the leaves were not affected by the DDT and had hatched. A second spray application using the same concentration resulted in 100% mortality without recurrence of the infestation.

MECHANICAL FACTORS AFFECTING APHID AND MITE POPULATIONS.—*Leaf pubescence.*—While observing the activity of aphids and mites on sprayed and unsprayed trees, it seemed significant that aphids and mites tended to become established on trees which possessed highly pubescent leaves to a much greater extent than on trees devoid of pubescence. Aphid and mite populations were compared on 10 trees possessing pubescent leaves with 10 trees without pubescent leaves. Five of the pubescent trees were infested with aphids, mites were present on seven, and four were infested with aphids and mites. Two of the non-pubescent trees were infested with aphids, three had mites, and one had aphids and mites.

Attaclay and Insecticide Residue on Leaves.—An experiment was conducted in which populations of black-margined aphids were compared on untreated trees, trees dusted with Attaclay, and DDT-sprayed trees. The purpose of the experiment was to determine whether the presence of an inert material such as attaclay on pecan leaves would have an effect similar to that obtained with DDT. For the first few weeks after treatment, populations of aphids were greatest on the DDT-sprayed surface, then a decline in numbers ensued. After several weeks, the aphids increased on both DDT- and Attaclay-treated leaves and surpassed populations on the untreated checks. The results indicated that the application of an inert substance, such as Attaclay, also produced large populations of aphids.

Mechanical factors such as leaf pubescence or surface residues appeared to influence significantly the development of aphid and mite infestations following the use of certain insecticides on pecan trees.

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SCIENTIFIC NOTES

Embryonic Starvation, an Explanation of the Defective Honey Bee Egg[1]

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In 1952 a queen breeder in a southern state sold over 40 queens, all of whose eggs were defective. This number of such queens was quite alarming from the standpoint of both buyer and seller. The occasional deposition of a defective egg may be quite common, but the complete sterility of the queen is relatively rare. Consequently Hitchcock (1956), who had received five of the queens, investigated the situation. He found, surprisingly, that these honey bee queens whose eggs all fail to hatch appeared otherwise healthy and normal.

The fact that the deposition of defective eggs is not limited to the honey bee but characterizes other hymenopterous species in which the occurrence of ovulation is dependent on external stimuli, and oviposition is highly specialized (Flanders 1950), is the basis of this discussion.

The viability of the hymenopterous egg, unlike the eggs of most insects, is not as a rule dependent upon its fertilization. In species in which the egg, upon its maturity, is well supplied with yolk, the viability of the egg can be markedly affected by its prolonged retention in the ovary. During the retention of the egg so much yolk may be extracted that the amount remaining is not sufficient for the development of a healthy embryo or larva.

In certain endoparasitic Hymenoptera, however, temporary inhibition of embryonic development is characteristic of the mature egg. Such inhibition, a result of the lack of yolk, ends when the egg receives nutrients directly from the body fluids of the host (Imms 1931). This inhibition of embryonic development permits the female to store her eggs in her oviducts. Ovisorption is thus precluded. In such a female ovulation is not necessarily externally induced and defective eggs presumably are rarely, if ever, deposited.

Species having some propensity for depositing defective eggs (*i.e.*, species in which ovulation is always externally induced) include endoparasites as well as ectoparasites. Contrary to Wigglesworth (1940) not all endoparasites produce eggs inherently poor in yolk.

Hymenopterous species known to deposit defective eggs include:

(1) *Bracon hebetor* Say, an ectoparasitic braconid which, according to Hase (1922), may deposit a high percentage of defective eggs when environmental conditions are optimum with respect to food and temperature. This observation was confirmed by Whiting (1940) who noted that the older the female the greater the proportion of defective eggs deposited.

(2) *Phaeogenes nigridens* Wesm., an endoparasitic ichneumonid that, in the absence of hosts, deposits defective eggs (Smith 1932).

(3) *Ephialtes extensor* Tasch, an ectoparasitic ichneumonid which was observed by Rosenberg (1934) to deposit eggs with one out of eight defective.

(4) *Melittobia chalybii* Ashm., an ectoparasitic chalcid which, when normally mated, deposits a large proportion of defective eggs (Schmeider 1938).

(5) *Euplectrus plathypenae* How., an ectoparasitic chalcid whose eggs may all fail to hatch if deposited on a host which is relatively unfavorable. Larvae that hatch from a few defective eggs fail to complete their development (Glenn L. Finney, unpublished preliminary observations).

(6) *Metaphycus luteolus* Timb., an endoparasitic chalcid which, when withheld from its host for 3 weeks, was observed by the writer to deposit one or more defective eggs.

(7) *Pimpla examinator* F., an endoparasitic ichneumonid whose deposition of defective eggs prompted Jackson (1937) to suggest that the deposition of defective eggs in ichneumonoid Hymenoptera could well be explained by such eggs having undergone regression while in the ovary. Hase (1922) had previously suggested that the defective egg resulted from decomposition of the oöplasm.

Certainly it is apparent that in certain Hymenoptera the composition of the egg may change in the interval between the moment of its maturity in the ovary and its deposition. The mechanism which can bring about such a change, supposedly characteristic of all the Hymenoptera in which ovulation is externally induced, is the process of ovisorption, a process which reduces the amount of yolk in the egg and whose effect is conditioned by the period the mature egg is retained in the ovary. The period of such retention is in part determined by the sensitivity of the gravid female to oviposition stimuli.

Consequently, we can conclude that the female which deposits defective eggs does so because of the prolonged retention of such eggs in the ovary, the result of an unusual delay in her response to ovulation stimuli.

Presumably this is the case in the honey bee. As pointed out by Hitchcock (1956) defective eggs have been occasionally observed in the brood comb of the hive over a period of many years. The queens that deposited such eggs behaved normally and oviposited in a regular manner. Cook (1910), who frequently received such queens for dissection, concluded that detecting the trouble with the egg was "a very difficult problem, if it is capable of solution at all." The eggs from such queens at the moment of deposition appear normal.

It was observed that a queen which deposited hatchable eggs early in life might in later life deposit only defective eggs. In this connection it should be noted that the correlation of available information on ovigenesis, ovisorption and oviposition in the queen indicates that (1) few, if any, eggs are deposited immediately on their maturity, (2) all or most of the deposited eggs have had some yolk extracted, (3) the amount of yolk reduction up to a certain point predisposes the embryo to become a worker and (4) any reduction beyond this point results either in the deposition of a defective egg or in its complete absorption prior to ovulation.

Hitchcock (1956), on the basis of his experiments and on the reports of earlier investigations, concluded that the production of defective eggs in the honey bee did not involve either pathogenic organisms or the ability of the nurse workers to care for the eggs and young larvae. In those queens which deposited only defective eggs the fat-bodies and ovaries were normal and the spermathecae contained viable sperm in abundance. The workers were able to recognize the defective eggs, for only a few of the brood cells containing such eggs were supplied with brood food. In one instance, however, 15 out of 867 of these cells were supplied with food and in one there was observed an apparently normal 3-day-old larva.

It is highly significant, as observed by Whiting (1940) in *Bracon hebetor*, and by Hitchcock (1956) in the honey bee, that embryonic development continues in each defective egg for a limited but variable period and that a few debilitated larvae may hatch from such eggs. Whiting (1940) pointed out that normal cleavage and blastoderm formation are not indicative of ultimate hatching. It is possible that among thousands of defective eggs one may occur which is normal and healthy. Hitchcock's studies have eliminated from further consideration most, if not all, the logical causes of the defective egg excepting that of embryonic starvation.

Studies by Mackenson (1951) on the reproduction of the honey bee show that the male larvae from defective eggs live somewhat

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longer than the female larvae from such eggs.

This difference in viability is to be expected because in hymenopterous species characterized by yolk-replete eggs, the minimum nutritional needs of the male are inherently less than those of the female.

In insects the size of the adult is a measure of the amount of food consumed during the larval stage so that the minimum size of the adult male when less than the minimum size of the adult female signifies that the larval male needed less food to complete its development. In the endoparasitic chalcid *Trichogramma* sp. the minimum size of the adult, 0.31 mm., is represented only by the male (Flanders 1931). In this respect, as pointed out by Salt (1936), the larval male is intrinsically superior to the larval female.

The maximum amount of food that the larval male can consume and then become adult is also less than the amount that can be consumed by the larval female. This is illustrated in *Pimpla examinator*, a solitary endoparasite of lepidopterous pupae. The larval female can "clean out" large hosts and thus provide the dry conditions needed for successful pupation. The larval male, however, when in such hosts, being unable to consume all the host's viscera, dies (Jackson 1937). The male of *Trichogramma* sp. may die under like circumstances. When the amount of yolk in the host egg is sufficient for the complete development of either 6 females or 10 males and females, the development of the solitary male is inhibited whereas that of the solitary female is not (Flanders 1935). It is significant that in the honey bee full-fed drone larvae in queen cells usually die instead of pupating (information from F. Ruttner of Austria). In such Hymenoptera the full-fed larval male is intrinsically inferior to the full-fed larval female.[2]

It appears, therefore, that the inherent size-range of the hymenopterous male is less than that of the reproductive female, a difference correlated with the haploid inheritance of the male.

The sexual differentiation in the mortality of the few larvae that hatch from defective eggs is presumably an effect of the greater susceptibility of the developing female to nutritional deficiencies. The fact that neither male nor female larvae from defective eggs can complete their development when provided with food is indicative of debilitating starvation during embryonic development.

The role of deficient embryonic nutrition in the development of nonreproductive females was postulated with reference to the bee, *Halictus malachurus* (Kby.), 12 years ago (Flanders 1946). Later, the lack of nutritive material for embryonic development was suggested as the causal factor in the occurrence of defective eggs in *Bracon hebetor* (Flanders 1953).

The production of defective eggs by otherwise healthy and normal honey bees, the possession of a mechanism for removing the yolk from the mature egg immediately prior to its deposition, the continuation of development for a limited period after deposition of the egg, and the longer survival period of the debilitated male relative to the debilitated female, all indicate embryonic starvation as the cause of the defective egg.

The occurrence of defective-egg-producing queens in the mating yards of the southern queen breeder was apparently eliminated through the changing of the drone breeding stocks (Hitchcock 1956). This can be explained on the presumption that the tendency in the queen toward prolonged retention of the ovarian egg is a recessive character. Crossbreeding experiments by W. C. Roberts involving queens completely defective-egg-producing, suggest that this phenomenon is hereditary (Hitchcock 1956). In any case its expression in the form of defective eggs is apparently enhanced by the aging of the queen.

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[2] In the females of the higher social Hymenoptera the minimum adult size attainable by the nonreproductive caste in contrast to the reproductive form may be less than that of the male. This is indicated by the observation of Mackenson (1951) that drone larvae, when developing in worker cells, often "die from lack of room."

Insects Caught in Light Traps with New Baffle Designs[1]

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Tests were conducted at State College, Pa., during the summer of 1957 to determine the value of two new types of baffles. All the traps in these experiments were equipped with 15-watt black-light fluorescent lamps and were built alike except for the construction of the baffles. The traps were hung in pairs on a crossarm 10 feet above the ground and placed so that the lamps were 3 feet apart. The positions of these traps were alternated every other day.

A cylindrical baffle, 20 inches long and 5 inches in diameter, made of two thicknesses of 0.04-inch cellulose acetate, was placed

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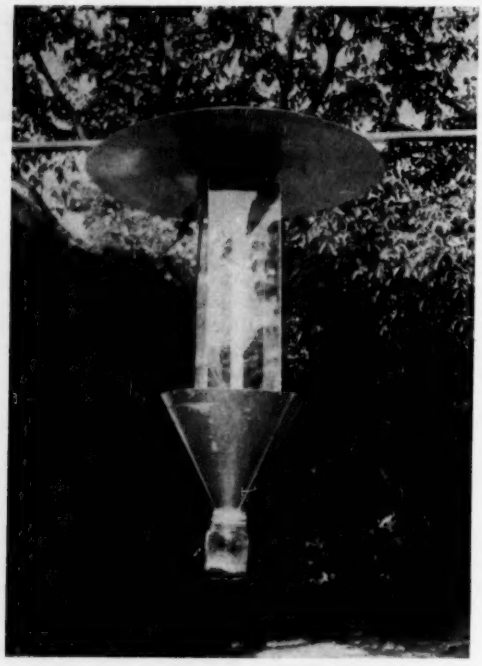

FIG. 1.—Trap with square prismatic plexiglas baffle.

around the lamp of one trap. The plastic material was fastened to two circular wooden blocks, one above the other below, allowing 3½ inches between the base of the cylinder and the edge of the funnel, for the insets to pass downward into the cyanide jar at the bottom of the trap. A prismatic baffle with four sides, each 20 inches long and 6 inches wide made of ⅛-inch plexiglas, fastened to two square wooden blocks, was placed around the the lamp of another trap. Ample clearance between the base of the baffle and the edge of the funnel permitted the insects to pass downward into the cyanide jar at the bottom of the trap. The new baffle designs were compared with 4, 20×5-inch intersecting, aluminum baffles of the standard Pennsylvania insect light trap.[2]

Two factors, that could not be separated under the existing circumstances, were (1) the shape and construction of the baffles, (2) the transmission of light, especially ultraviolet, by the plastic materials. Information supplied by the Rohm & Haas Co. indicates that from 75 to 90% of the ultraviolet rays and slightly more than 90% of the visible rays are transmitted by the plexiglas used. Between 3000 and 3200 Ångstroms, 75% of the rays are transmitted and between 3200 and 4000 Ångstroms 90% are transmitted. Henry J. Enos, a biochemist of the Pennsylvania State University, ran some tests with the Coleman spectrometer on cellulose acetate. Between 3300 and 4000 Ångstroms, one thickness of 0.04-inch material transmitted 71%, two thicknesses 55% and three thicknesses 40.5% of the ultraviolet rays.

The Nemocera included Culicidae, Simuliidae, Rhyphidae and Tipulidae, but they were not taken in very large numbers. Miscellaneous midges included Psychodidae, Mycetophilidae, Cecidomyidae, and Chironomidae. These species were always taken in considerable numbers but never separated into families.

The insects captured are listed in table 1. It is evident that no advantage was gained by using a cylindrical or square prismatic baffle. There was considerable reduction in the number of insects caught in the two traps with plastic baffles. The reduction in the case of the trap with the plexiglass baffle was far less than with the trap having the cellulose acetate baffle. This may indicate that the reduction in general may be due to the decrease of ultraviolet transmitted rather than the construction of the baffles. In any case, the results were negative.

Table 1.—Insects caught in light traps with different baffle designs.

Insects	July 21–30		Aug. 11–24	
	Lamp Between 4 Intersecting Aluminum Baffles	Lamp Within Cylindrical Acetate Baffle	Lamp Between 4 Intersecting Aluminum Baffles	Lamp Within Square Prismatic Plexiglas Baffle
Nemocera	92	60	107	61
Miscellaneous Midges	2,398	1,354	2,317	1,419
Brachycera	721	427	271	123
Carabidae	599	231	110	61
Staphylinidae	468	288	136	172
Scarabaeidae	177	59	43	44
Miscellaneous Coleoptera	2,828	1,585	2,570	1,921
Aphididae	563	225	263	138
Cicadellidae	31,920	8,433	1,215	1,008
Miscellaneous Homoptera	248	113	30	52
Miridae	759	365	212	158
Miscellaneous Hemiptera	25	29	73	34
Pyralidae	70	73	147	103
Crambidae	357	122	813	654
Geometridae	21	24	34	34
Noctuidae	420	268	389	313
Arctiidae	194	109	16	21
Tortricidae	146	107	199	216
Codling moth	56	27	39	24
Macrolepidoptera	102	48	66	40
Microlepidoptera	862	310	193	147
Macrotrichoptera	1,585	689	709	349
Microtrichoptera	243	100	252	192
Hymenoptera	553	337	921	402
Miscellaneous insects	251	81	160	73
Totals	45,658	15,464	11,285	7,759
Percentages	74.7	25.3	59.2	40.8

[2] Frost, S. W. 1957. The Pennsylvania insect light trap. Jour. Econ. Ent. 50: 287–92.

Notes on Rearing the Moths, *Cisseps* (*Scepsis*) *fulvicollis* (Hbn.) and *Ctenucha virginica* (Charp.), in the Laboratory[1]

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Cisseps (*Scepsis*) *fulvicollis* (Hbn.) (Lepidoptera, Amatidae), a common moth in the eastern U. S., is easy to rear in the laboratory and has a rather short life cycle, only about 5 weeks. It seems worth calling this possibly useful experimental animal to the attention of those whose experiments might be done with a moth which can be easily and cheaply cultured. In our laboratories, this species was reared through 11 generations, from Aug. 7, 1955 until Jan. 4, 1957, at which time the culture was discontinued voluntarily.

Adults were housed either in cubical screening cages (30 cm. on a side) or in wide-mouth gallon jars covered with plastic fly screening. They were given water and sugar-water (approx. ½ M sucrose solution): in the cages by inverting bottles of the fluids covered with cheese-cloth on the tops, and in the gallon jars by sprinkling the walls of the jars with water and furnishing sugar-water in bottles inverted on the tops.

In the cages, for the first few generations, eggs were collected on strips of mosquito netting or cheese-cloth laid on the tops; the moths oviposited through the screening. It was thus unnecessary to open the cages and risk escape. Later we found that adhesive cellulose tape (Scotch or Texcel) laid sticky side down on the tops of the cages was preferred by the moths for oviposition. The eggs were fastened in neat rows corresponding with the meshes of the screening, and all were oriented similarly. This method of collection might be useful for embryologists interested in having eggs oriented for study. We also tried electrically charged cellulose sheets (Saranwrap), and these too proved

[1] Accepted for publication July 28, 1958.

excellent. In the gallon jars, the moths were supplied with fresh grass blades for oviposition. The adults began to deposit eggs 3 to 6 days after emergence, and they lived for 20 to 30 days.

The eggs laid by the adults in cages were transferred, with the material on which they were deposited, to wide-mouth gallon jars for hatching. When the adults were already in gallon jars, this step was eliminated. The eggs hatched in 5 to 8 days at laboratory temperatures (18° to 23° C.).

The larvae were fed on grass blades (Edwards 1889, Holland 1916), furnished as needed from outdoor cuttings. Two species of grass were used, orchard grass (*Dactylis glomerata*) and timothy (*Phleum pratense*), but other species would undoubtedly be suitable. From April until December these were readily available from fields or roadsides. The leaves were cut and stored in plastic bags in a refrigerator until needed. It was thus possible to obtain enough for a few weeks within a short time. During the winter more trouble was encountered in getting fresh grass outdoors. Only the bases of the leaves which were protected by the frozen ends were useful. We had to cut much more than in the summer, cut close to the ground line and then trim away the dead parts before storage. It was possible, however, to get enough for 400 to 600 larvae for 2 to 3 weeks and to trim and sort the good parts in about 3 hours. If one had a small greenhouse, it would be easy to raise grass during these months.

Lettuce and moistened timothy hay were tried as substitutes for fresh grass, but they proved useless. Frozen grasses were also tried, and some larvae grew on them, but much more slowly than on fresh grass. Few reached adulthood (in one test, 2 out of 100).

The larvae were reared in wide-mouth gallon jars, where grass was supplied as needed. About 150 fully grown larvae and more early instar larvae could be conveniently kept in one jar. If feeding was carefully managed, it was possible to bring these through to pupation without transfer. If, however, there was much inedible material in the grass, or too much was given repeatedly, it was necessary to move the larvae to new jars when they were about three-quarters grown.

The length of larval life, at laboratory temperatures (18° to 23° C.), was 22 to 30 days. In two cases, jars with larvae were kept at about 15° C., and 35 to 50 days were required. The time needed for development was also extended if the food supply was low.

For pupation, the larvae produced a flimsy silken cocoon on the sides of the jars or on the grass. The pupae were removed from these to paper troughs in cages or in gallon jars for emergence. At laboratory temperatures, adults emerged in 6 to 10 days. An attempt was made to keep a stock of potential adults on hand by refrigerating the pupae at 4° to 6° C., but, after 14 days at these temperatures, only a few adults emerged when the pupae were returned to the warmth, and these failed to oviposit. After 20 days or more in the cold, no adults emerged.

The first few generations were reared for experimental purposes, and therefore care was taken to assure good conditions. The last ones, however, were tended only when time could be found from other activities. Even with this semi-neglect, production was good. This moth, therefore, can be recommended as very easy to rear. Furthermore, the life cycle is relatively short—33 to 40 days from egg to adult at ordinary temperatures. With higher temperatures, this could undoubtedly be reduced to less than 30 days. The moths are of reasonable size—about 15 cm. body length—and thus suitable for physiological or toxicological experiments.

At the same time, and with the same methods, we also reared *Ctenucha virginica* (Charp.), another moth in the same family. This insect, however, has a much longer life cycle; only two generations were reared from June 20, 1955 until May 24, 1956. The eggs hatched in 4 to 8 days, the larval period was 140 to 200 days, the pupal period 16 to 20 days. Thus the total time from egg to adult was 160 to 225 days at laboratory temperatures. The adults lived for about a month in captivity and began oviposition within a day or two after emergence.

Ctenucha virginica is a little larger than *Cisseps fulvicollis*, and the larval instars can be easily distinguished by differences in appearance. The long life cycle, however, would seem to make it inconvenient for rearing in the laboratory.

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Tests with a New Organophosphorus Compound (Dowco 109) against Cattle Grubs in Oregon[1]

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Research on and for compounds with systemic activity against insects attacking livestock has been greatly accelerated during the last 3 or 4 years. This has been especially true of the research on cattle grubs, *Hypoderma lineatum* (De Vill.) and *H. bovis* (L.), with the result that considerable progress has been made. The findings on Dow ET-57 (Trolene) by Roth & Eddy (1957) and McGregor & Bushland (1957), and on Bayer 21/199 (Co-Ral) by Brundrett *et al.* (1957) represent two of the more recent and important developments. Both materials are phosphorus compounds, ET-57 being more effective against grubs as an internal treatment and Bayer 21/199 as a spray application. Neither material has a very wide margin of safety, and both possess certain other limitations. The ideal grub systemic, therefore, has not been discovered.

A new material that has shown promise when either administered orally or applied as a spray is Dowco 109 (*O*-(4-tert-butyl-2-chlorophenyl)*O*-methyl methylphosphoramidothioate).[2] This material was available last year to the extent that several workers were able to conduct limited tests with it during the 1957–58

[1] Accepted for publication September 8, 1958.

[2] Product of Dow Chemical Company, Midland, Michigan.

Table 1.—Control of cattle grubs with oral treatments of Dowco 109.

Treatment Mg./Kg. (1957)	No. of Animals	Examination in— February: Grubs per Animal	February: Per Cent Control	March: Grubs per Animal	March: Per Cent Control	April: Grubs per Animal	April: Per Cent Control
November 26							
4	15	3.7	67	3.2	73	0.7	91
6	11	2.0	82	1.5	87	0.5	94
Control	27	11.2	—	11.9	—	8.3	—
October 18							
10	10	0	100	0.7	86	0.6	91
Control	10	6.0	—	5.0	—	6.8	—
November 13							
15	20	0.8	74	—	—	0.2	93[a]
Control	18	3.4	—	—	—	2.8	—
December 9							
20	12	0	100	0.2	99	0.2	97
Control	14	24.4	—	18.0	—	8.0	—
December 4							
20	13	0	100	0	100	0.1	98
Control	14	11.3	—	10.5	—	4.5	—
October 18							
25	10	0	100	0.3	94	0.1	99
Control	10	6.0	—	5.0	—	6.8	—
November 13							
25	10	0	100	—	—	0.1	96[a]
Control	18	1.6	—	—	—	2.3	—

[a] Examinations made in May.

Table 2.—Control of migrating cattle grubs with sprays containing 0.75% of Dowco 109.

Treatment Mg./Kg. (1957)	No. of Animals	Examination in— February: Grubs per Animal	February: Per Cent Control	March: Grubs per Animal	March: Per Cent Control	May: Grubs per Animal	May: Per Cent Control
November 22 (2)[a]	23	1.2	84	1.3	71	0.2	80
Control	23	8.1	—	4.1	—	1.0	—
November 13 (2)	16	0	100	—	—	0.7	71
Control	18	1.6	—	—	—	2.7	—
December 9 (4)	13	0	100	0.1	99	0	100
Control	14	24.4	—	18.0	—	8.0	—

[a] Figures in parentheses represent average number of quarts per animal.

grub season. The tests made by the authors are reported herein. The animals were treated in the fall and examined for grubs in February, March, and April or May.

Most of the test animals were weaners or short yearling Herefords weighing from 400 to 500 pounds. For treatment they were divided at random and ear-tagged. Those used as controls were not separated from the treated ones. Most of the animals used as controls were treated with either DDT or rotenone for louse control, mainly to satisfy the owner. Previous data, however, have indicated that sprays of these materials do not act systemically against grubs.

For the oral treatments the animals were weighed on a portable scale just before the material was administered. Some of the herds were treated with the technical material in boluses and some in gelatin capsules, both forms being administered with a balling gun. The amount of material given was weighed to insure accuracy of the dose.

The results of these treatments are summarized in table 1. Examinations were made three times to coincide with the peak incidence of the two species. Practically all of the grubs appearing in March are *lineatum*, whereas those in April are *bovis*. The March records represent both species since there usually is an overlapping of the two at this time. As can be seen from the data, fair to excellent control was obtained at rates from 4 to 15 mg./kg., and at 20 or 25 mg./kg. the control was 94 to 100%.

Since the treatments described above were given before the grubs appeared, a single yearling Guernsey steer having 22 encysted second- and third-instar larvae was treated at 25 mg./kg. Dead larvae were noted on the third day, and all were dead after 14 days. The material appeared to be more effective against large third-instar larvae than ET-57.

The sprays were applied with a power sprayer at 250 to 300 pounds operating pressure, 2 or 4 quarts per animal. Both the oral and spray treatments were made before the grubs appeared in the backs of the animals.

The results of the spray treatments are presented in table 2. The 0.75% spray gave fair to excellent control. Four quarts per animal was distinctly better than 2 quarts, which is in line with the authors' previous findings with sprays of Bayer 21/199 (unpublished data).

Since Dowco 109 appears to be as effective as Bayer 21/199 as a spray or ET-57 as an oral treatment, it is more versatile as a systemic in the control of cattle grubs.

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Systemic Activity of Sevin in Control of *Ornithonyssus sylviarum* (C. & F.)[1]

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The recent successful use of systemic insecticides for control of livestock pests has received wide acclaim. This is amply justified in the case of systemic insecticides for control of the cattle grub, since this approach represents the only practical, effective method yet devised to control the pest. Similarly it would appear that if systemic insecticides could be applied orally to poultry they would possess a singular advantage over currently used insecticides; application in the feed would eliminte individual, or even flock spraying, dusting or dipping, with attendant drops in egg production.

Preliminary tests have been conducted to determine the systemic activity of N-methyl-1-naphthyl carbamate (Sevin[2]) in the control of the northern fowl mite, *Ornithonyssus sylviarum* (C. & F.) on White Leghorn hens. A wettable powder of 50% Sevin was added to laying mash and throughly integrated in a mechanical mixer. Infested birds were treated in both laboratory and limited field tests. An arbitrary scale of infestation density was used as follows:

very light—less than five mites
light—five to numerous mites
moderate—numerous mites, greying feathers
heavy—many mites, blackened feathers

Results.—In a preliminary laboratory screening test one heavily infested hen was fed for 5 days on feed containing 3,000 p.p.m. of actual Sevin. The daily feed consumption was 140 grams, which corresponded to approximately 245 mg. of technical Sevin per kg. of body weight daily. Results are given under Experiment 1, table 1. The same table gives the results of three subsequent field experiments in which time of application and amount of actual Sevin consumed varied. During the observation periods following treatment indicated in table 1, birds in Experiments 2, 3 and 5 were continuously exposed to reinfestation from adjacently caged, infested birds.

In addition to the tests summarized in table 1 another group of five moderately infested hens was offered feed containing 3,000 p.p.m. Sevin, but the feed was removed, by error, on the first day when the birds had consumed only an average of 78 mg. of technical Sevin per kg. of body weight. After 40 hours one of the birds had retained a light infestation, two remained with very light infestations and two were free of live mites. The birds were then given another ration of the same feed which they ingested in an amount equivalent to approximately 150 mg. of Sevin per kg. of body wt. They were all free of mites 3 days after being offered the second treated ration. No recurrence of infestation was noted within 10 days.

That the systemic activity of Sevin may be selective in nature was demonstrated in an experiment in which 40 specimens from a laboratory culture of *Cimex columbarius* Jenyns were allowed to feed for 3 days on hens having as a sole food source a diet containing 3,000 p.p.m. of Sevin. Neither mortality nor ill effects on the bugs was produced.

Apparently feed containing 3,000 p.p.m. of Sevin has no repulsive taste or odor to hens since they fed on it without hesitation. A drop in egg production of approximately 10% for a few

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[2] Manufactured by the Union Carbide Chemicals Co.

Table 1.—Effect on the northern fowl mite of Sevin applied in feed to White Leghorn hens.

Number of Birds	Days Medicated Feed Offered	Sevin in Feed (p.p.m.)	Total Sevin Ingested (mg./kg. body wt.)	Pretreatment Infestation	Infestation on Days After Treatment Was Initiated				
					1	2	5	7	21
				Experiment No. 1					
1	5	3,000	1,225	heavy	—	none	none	—	—
				Experiment No. 2					
2	4	3,000	740	moderate	none to light	none	—	—	none
5	0	0	0	moderate	moderate	moderate	—	—	heavy
				Experiment No. 3					
5	1	3,000	175	moderate to heavy	—	none	—	—	none
8	0	0	0	light to heavy	—	moderate to heavy	—	—	moderate to heavy
				Experiment No. 5					
2	2	3,000	270	heavy	—	none	—	none	—
4	1	3,000	140	heavy	—	none	—	none	—
4	1	1,500	68	heavy	—	none to light	—	none to very light	—
2	0	0	0	heavy	—	heavy	—	heavy	—

days was observed in one case but it could not be attributed to the treatment with certainty. No ill effects of the treatment on hens was observed with the exception of one bird that died after consuming approximately 270 mg. of Sevin per kg. of body weight over a 48-hour period. All previous observations indicate that this death was not due to the feeding of Sevin. For further confirmation of this point five hens were fed in the laboratory on feed containing 3,000 p.p.m. Sevin for 8 days without evident symptoms. They ate during this period a total of 920 mg. of technical material per kg. body weight, an average daily intake of 115 mg. Sevin per kg. body weight. The greatest intake of Sevin for an individual bird was 1225 mg. per kg. body weight, ingested over a 5-day period with no ill effects.

Carbon Disulfide Fumigation of the European Chafer[1]

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Fumigation with carbon disulfide is authorized by the Plant Pest Control Division as a basis for the certification of soil subject to infestation by the white-fringed beetle and against the Japanese beetle. The respective dosages are 2 and 1 pound per cubic yard, with an exposure of 48 hours at temperatures above 40° F. Experiments were conducted at Geneva, N. Y., during 1955–57 to determine the effectiveness of this fumigant in eliminating the soil-inhabiting stages of the European chafer, *Amphimallon majalis* (Raz.), to satisfy the requirements of the Federal and State quarantines against this insect.

TESTING PROCEDURE.—Wooden boxes 18 inches square and 18 inches deep were lined with plastic sheeting or kraft paper as a vapor barrier. Each box was filled with friable soil and chafers in one stage were distributed throughout the mass. The fumigant was then poured into a hole 1 inch wide by 2 inches deep in the center of the soil surface. The entire surface of the soil was then covered immediately with plastic sheeting or wet newspaper. This treatment approximates the application of the fumigant to an 18-inch layer of soil at 18-inch intervals. A dosage of 44 ml. per box was equivalent to 1 pound per cubic yard of soil.

After an exposure of 48 hours the various stages of the chafer were transferred to untreated soil to determine their responses. Soil temperatures in the boxes were determined just before and after the exposure period.

TESTS AGAINST EGGS.—Groups of 100 fertile eggs distributed in approximately 50 grams of soil were placed in bags made with a single layer of cheesecloth. Each bag was in turn inserted in a cylinder of 14- by 16-mesh metal screen to protect the eggs from being crushed. Either two or four bags were buried in each box of soil and exposed to dosages of carbon disulfide ranging from $\frac{1}{4}$ to $1\frac{1}{4}$ pounds per cubic yard. Soil temperatures ranged from 66° to 74° F. Observations were made for 3 weeks, during which time they either hatched or decomposed.

TESTS AGAINST LARVAE.—One hundred third-instar larvae were distributed throughout the soil mass, or four perforated-metal cages each containing 25 larvae were buried in each box of soil. Both methods of infesting gave comparable results. Since this is the only stage prevailing during the colder periods of the year, temperature-mortality relationships with a constant dosage of 1 pound of fumigant per cubic yard of soil were determined between 32° and 60° F. The larvae were subjected also to dosages from $\frac{1}{4}$ to 1 pound per cubic yard at temperatures above 45°. Reactions of larvae to the treatments were determined immediately after removal from the treated soil and again 24 hours later.

TESTS AGAINST PUPAE.—Groups of 25 pupae were placed in perforated-metal cages with soil. Two cages were buried in each box of soil and exposed to dosages from $\frac{1}{4}$ to 1 pound per cubic yard at temperatures above 60° F. Pupal mortalities were determined at the end of the exposure period and again 24 hours later.

TESTS AGAINST ADULTS.—Groups of 25 males and 25 females were confined in separate screen cages. Four cages of each sex were buried in each box of soil and exposed to dosages from $\frac{1}{4}$ to 1 pound per cubic yard at temperatures above 62° F. Mortalities were determined immediately after removal from the treated soil and again 48 hours later.

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[2] In cooperation with the Department of Entomology, New York State Agricultural Experiment Station, Geneva, N. Y.

Table 1.—Mortality of different stages of the European chafer exposed to carbon disulfide in soil.

Pounds of carbon Disulfide per Cubic Yard	Eggs		Larvae		Pupae		Adults[a]
	Number Exposed	Per Cent Mortality	Number Exposed	Per Cent Mortality	Number Exposed	Per Cent Mortality	Per Cent Mortality
0.25	400	21.3	300	65.3	—	—	99
.5	1000	97.8	400	84.5	50	62	100
.75	1000	99.6	300	98.0	100	100	
1	1000	100	400	100.0	100	100	100
1.25	1000	100					
0 (Check)	1000	11.5	400	2.4	100	18	30

[a] 200 of each sex exposed to each dosage.

Table 2.—Effect of temperature on third-instar European chafer larvae exposed to 1 pound of carbon disulfide per cubic yard of soil.

Minimum Soil Temperature (° F.) During Exposure		Number of Larvae	Per Cent Mortality
Beginning	End		
32	32	100	61.0
42	38	300	83.3
45	40	300	100.0
60	50	300	100.0
60	55	400	100.0
(Check (untreated))		400	2.4

Results (Tables 1 and 2).—There was only slight ovicidal action with ¼ pound of carbon disulfide per cubic yard of soil, but high mortality was obtained with ½- and ¾-pound dosages. The 1-pound dosage eliminated the eggs and third-instar larvae at temperatures above 45° F. All pupae were eliminated by the ¾-pound dosage, and all adults by ½-pound. There was no sexual difference in susceptibility to the fumigant.

In view of these results, carbon disulfide is recommended for the treatment of potting soil subject to infestation by the European chafer.

Laboratory Tests with Insecticides against *Drosophila melanogaster*[1]

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Drosophila flies are troublesome pests to processors of fruits and vegetables. Contamination occurs in the fields, in transit, and at the processors' plant, when the flies deposit eggs in the cracks and crevices of the broken skin of the fruit or vegetable.

In previous experiments fair to poor control of drosophila has been obtained with pyrethrum, Dilan, TEPP, Lethane, DDT, allethrin, lindane, dieldrin, TDE, chlordane, heptachlor, aldrin, Dipterex, and malathion (Ditman *et al.* 1936, Warner 1951, Ditman & Bickley 1952, Collins 1953, Pepper *et al.* 1953, Michelbacher & Middlekauff 1954, Bickley *et al.* 1955, Mason 1956, Stombler *et al.* 1957).

In 1955, 1956, and 1957 laboratory screening tests were conducted at Logan, Utah, to furnish information on the effectiveness of about 40 insecticides at various strengths in killing adults of *Drosophila melanogaster* Meig.

A 6-inch painted wooden pot label was dipped into emulsion or suspension to a depth of about 4 inches, and as soon as it was dry was placed at an angle in a ½-quart cardboard carton with 25 drosophila adults. The carton had cheesecloth side windows and top, and contained food for the flies. The mortalities in 4 and 24 hours were recorded. The label was then removed from the carton and suspended on a rope outside the laboratory window for exposure to sunlight and wind. After various intervals outside, the label was again tested for 24 hours against drosophila in the cardboard carton. The tests were replicated four times.

In preliminary tests wettable-powder suspensions became ineffective much earlier than emulsions. The results reported herein were with emulsions except as otherwise indicated.

In order to standardize the results, the Canton strain of *Drosophila melanogaster* was used. However, preliminary comparison with a local wild strain showed almost identical results.

The following insecticides at 2% gave less than 50% mortality after 24 hours: DDT, pyrethrum, rotenone, Chlorthion, ryania, Guthion, Perthane, toxaphene, piperonyl butoxide, TDE, Sevin (1-naphthyl-N-methyl carbamate), Chlorobenzilate, Strobane, methoxychlor, Karathane, allethrin, and thiourea. Twenty-one

Table 1.—Per cent mortality of adults of *Drosophila melanogaster* exposed to surfaces (dipped in 2% emulsions of various insecticides) for 4 hours and for 24 hours ending at indicated days after treatment.

Insecticide	4 Hours	1 Day	3 Days	10 Days	17 Days	22 Days	30 Days
DDVP	100	100	100	100	96	96	94
Heptachlor	100	100	100	100	100	98	98
Dow ET-57	100	100	100	100	100	94	33
Endrin	85	100	100	100	100	92	92
Dieldrin	87	100	100	100	100	100	98
Isodrin	100	100	100	100	92	84	60
California Spray 4355[a]	100	100	100	100	96	66	33
Parathion	70	100	100	96	85	53	—
Malathion	99	100	100	94	92	47	—
Diazinon	100	100	100	93	80	72	5
Aldrin	100	100	100	100	86	47	—
Dicapthon	100	100	100	100	78	57	—
Chlordane	100	100	100	87	47	—	—
Trithion	100	100	93	92	70	25	0
Lindane	100	100	100	90	65	0	—
Thimet	100	100	92	90	—	—	—
BHC	100	100	100	49	43	0	—
Am. Cyanamid 12008	100	100	96	27	0	—	—
Dipterex (SP)	100	100	96	4	—	—	—
TEPP	96	100	19	—	—	—	—
Demeton	87	100	65	—	—	—	—

[a] 1,2-Dibromo-2,2-dichloroethyl dimethyl phosphate.

of the materials tested at 2% showed 100% mortality after the first 24 hours. These materials and the duration of their effectiveness are shown in table 1. Malathion and Diazinon, both of low mammalian toxicity, were effective for about 2 weeks, lindane and BHC for 7 to 10 days, and heptachlor for 30 days.

In further tests malathion and Diazinon at 1% were effective for 7 to 10 days, and at 0.5% for 3 and 7 days, respectively. Lindane and BHC at 1% were effective for 3 to 5 days. Heptachlor at 1% was effective for 21 days, at 0.5% for 10 days, and at 0.25% for 7 days. These insecticides thus have promise for field use in control of drosophila.

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[2] In cooperation with College of Agriculture, Utah State University.

Further Tests with BHC Emulsion Sprays to Keep Boring Insects Out of Pine Logs in Massachusetts[1]

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After spray applications of BHC and lindane emulsifiable liquids diluted in water were found to give excellent protection of unseasoned pine logs against bark and wood boring insects at Sunderland in the Connecticut Valley area of Massachusetts (Becker 1955), it seemed desirable to repeat the experiments under the insect and weather conditions existing elsewhere in the state. Consequently, in 1955, tests were conducted in the hills at Pelham, near the coast at East Sandwich on Cape Cod, and also at Pembroke, a little north of the Cape. Repeated forest fires and occasional hurricanes have often resulted in large populations of pine bark and wood boring beetles in recently killed trees and unseasoned logs on Cape Cod and vicinity.

EXPERIMENTAL PROCEDURE.—Unseasoned pine logs were sprayed once in the spring with a commercially prepared BHC emulsifiable liquid concentrate which had been diluted in water to 0.2 or 0.4% gamma isomer content by volume. The applications were made before or just as the beetles started to attack the logs. The entire surface of each log was sprayed until the liquid began to run off. Each treatment involved between 127 and 295 square feet of bark area. The logs were left in partial shade in pine woods until autumn when the entire bark area on all test logs was examined for insects and insect damage. Insects were identified by specialists in the Insect Identification and Parasite Introduction Laboratories, Entomology Research Division, U. S. Department of Agriculture.

At Pembroke, white pine trees, *Pinus strobus* L., felled by the hurricane in September 1954 were sawed into logs and sprayed near May 1, 1955 just as the bark beetle, *Pityogenes hopkinsi* Sw., started to attack. The logs were 5 to 10 inches in diameter.

In Pelham, logs from live, winter-felled, eastern white pines, *Pinus strobus* L., and red pines, *Pinus resinosa* Ait., were sprayed May 3 when the bark beetles, *Dendroctonus valens* Lec. and *Pityogenes hopkinsi* Sw. were starting to attack. These logs were 4 to 14 inches in diameter.

At East Sandwich, live healthy pitch pines, *Pinus rigida* Mill., were felled on June 21 and the resulting logs sprayed the same day before any beetles attacked then. These logs were 3 to 10 inches in diameter.

RESULTS.—At all three localities, one application of the 0.4% gamma isomer spray in the spring resulted in 97.5 to 100% protection throughout the entire summer against all *wood boring* insects. Protection against various *insect pests of the bark and cambial region* varied from 99.2 to 100%, except in one instance

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Table 1.—Insect galleries per square foot of bark area in pine logs sprayed with BHC emulsions.

	Pelham Tests Eastern White and Red Pines			East Sandwich Tests Pitch Pine			Pembroke Tests Eastern White Pine	
	0.4% Gamma Isomer	0.2% Gamma Isomer	Unsprayed Checks	0.4% Gamma Isomer	0.2% Gamma Isomer	Unsprayed Checks	0.4% Gamma Isomer	Unsprayed Checks
	Square Feet of Bark Area in Each Treatment							
Insects	161	150	168	127	138	156	295	204
			In wood					
Round-headed borers: *Monochamus* sp.	0	0.007	0.125	0	0	0.940	0	0.054
Others (*Asemum moestum* Hald., *Xylotrechus sagittatus* (Germ.), *Arhopalus obsoletus* (Rand.), *Astylopsis sexguttata* (Say), *Clytus marginicollis* Cast.)	0	0	0.423	0	0	1.840	0	0.338
Flat-headed borers: *Chrysobothris* sp.	—	—	—	0	0	0.013	0	0.407
Ambrosia beetles: *Gnathotrichus materiarius* Fitch	0.006	0.040	0.250	0	0.007	6.340	0	—[a]
			In bark or cambial region					
Weevils: *Pissodes approximatus* Hopk.	0.043	0.333	5.440	0	0	0.692	0.122	0.260
Round-headed borers: *Stenocorus lineatus* (Oliv.)	0	0	Trace	—	—	—	0	0.495
Bark beetles:[b] (*Dendroctonus valens* Lec., *Hylurgops pinifex* (Fitch), *Orthotomicus caelatus* (Eichh.), *Ips calligraphus* (Germ.), *Ips pini* Say, *Pityogenes hopkinsi* Sw., *Crypturgus atomus* Lec.)	Trace	0.2%	35.2%	0	Trace	68.5%	Trace	85.0%

[a] Common but not counted.

[b] Bark beetle infestations were measured by the percentage of bark area covered by their brood galleries.

(at Pembroke) against the bark weevil, *Pissodes approximatus* Hopk., when only 53.0% protection resulted.

Protection provided by the 0.2% gamma isomer spray, used only at Pelham and East Sandwich, varied from 84.0 to 100% against various *wood boring species*. Against *insect pests of the bark and cambial region*, protection varied from 93.9 to 100%. These percentages are based on a comparison of the number of insect galleries per square foot of bark on the sprayed and unsprayed logs or, in the case of bark beetles (Scolytidae), on a comparison of the percentage of bark area covered by the galleries, as listed in table 1.

The results of these pine log spraying tests with BHC emulsions, under various intensities of insect attack at three widely separated locations in Massachusetts, substantially confirm the successful results obtained earlier with the same concentrations of this insecticide in the Connecticut Valley.

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Influence of Water Phase of Oil Spray on Photosynthesis in Eureka Lemon and Bearss Lime Leaves[1]

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Relation of oil type and deposit to the effects of highly refined spray oils on photosynthesis in leaves of Eureka lemon and Bearss lime has been reported by Riehl & Wedding (1959). Emulsifiers were dissolved in the oils to prepare the emulsive formulation. The formulated oil was applied directly to the leaf of the one-leaf test plant by means of a spray tower. However, the thorough coverage required for citrus pest control without exceeding the amount of oil deposit that can be used safely on the tree has, until now, dictated the use of dilute aqueous spray mixture.

The experiments reported here were conducted to compare direct application and application in aqueous emulsion so that the factor of primary consideration would be any influence that the water phase of the oil spray might have on the effects of several spray oils on photosynthesis in leaves of Eureka lemon and Bearss lime.

Materials and Methods.—Descriptions of the single-leaf potted plants and details of the experimental procedures may be found in the report by Riehl & Wedding (1959). The several oils used in the experiments reported here were taken from the same stocks as those used in the preceding work by Riehl & Wedding (1959; see table 1 for selected properties of the oils). The alphabetical designation used in that report for given oils will be used here. Each of the oils was prepared as a emulsive-type spray oil by the addition of a mixture of oil-soluble emulsifiers (two parts No. 1159 and one part No. A023; made by E. F. Houghton and Co., 303 W. Lehigh Ave., Philadelphia 33, Pa.) at a concentration equivalent to 13 ml. of the mixture of emulsifiers per gallon of oil.

The formulated emulsive oil was applied either directly or in aqueous emulsion by means of a vertical spraying tower (ten Houten & Kraak 1949) with a precision nozzle (loaned to us by the Shell Oil Company). A homogeneous emulsion of one part of the emulsive oil and one part tap water was prepared and maintained during the application by recycling the mixture through a small electric centrifugal gear pump connected with the reservoir tank attached to the nozzle of the spray tower. The reservoir tank was employed in the same manner as those of the several types shown by ten Houten & Kraak (1949). A rheostat was used to regulate the speed of the gear pump to that just sufficient to maintain the emulsion. The gravimetric method was used to measure the deposits; the weights were taken as soon as possible, and the factor for water evaporation was applied to the emulsion deposits. The deposit of actual oil in the emulsion, derived by calculation, was used for the calculations of regressions of percentage of inhibition of photosynthesis on oil deposit.

Discussion of Results.—Derived values obtained by cal-

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Table 1.—Comparisons of effects of application of various petroleum spray oils in aqueous emulsion or directly on the per cent inhibition of photosynthesis in citrus leaves.

Oil[a]	Days After Application	Calculated Per Cent Inhibition of Photosynthesis ($Y \pm s_y$)					
		Micrograms Oil per Sq. Cm. Applied in Aqueous Emulsion			Micrograms Oil per Sq. Cm. Applied Directly		
		150	300	500	150	300	500
		Bearss Lime					
A	2	7.4± 2.8			3.4±3.5		
	7	−4.2± 6.3			− 3.5±5.9		
	21	−5.3± 4.3			2.5±2.1		
I	2	2.7±11.2			5.4±3.4		
	7	−4.5± 8.2			0.3±3.9		
	21	3.4± 6.3			4.4±3.3		
		Eureka Lemon					
G	2		17.0±5.4	40.2±6.6		11.8±1.9	49.4± 2.7
	7		17.2±2.8	41.8±2.4		15.0±3.4	48.0± 4.7
	21		1.2±6.2	8.8±5.3		10.9±2.2	16.1± 3.0
H	7		18.1±3.3	32.3±2.4		12.8±2.8	33.4± 3.7
	21		− 9.8±4.4	1.6±3.3		7.1±3.6	6.1± 4.7
J	2	1.2± 2.9			− 2.7±4.8		
	7	−7.8± 5.1	36.2±3.3	48.4±4.5	−13.3±4.2	20.6±3.0	44.2± 4.2
	21	−5.8± 5.3	26.6±3.9	47.2±5.3	4.6±5.5	10.2±6.8	45.6±10.3

[a] For properties of the oils, see table 1, Riehl & Wedding (1959).

culations of regressions of percentage inhibition of photosynthesis on oil deposit are listed in table 1 for the selected deposit of 150 for the deposit range of 100 to 300 μg. oil/sq. cm. of leaf surface and for the selected deposits of 300 and 500 for the range of 300 to 600 μg. (Riehl & Wedding 1959).

No real differences are apparent in comparisons of the respective calculated values of mean percentage of inhibition of photosynthesis for the given oils applied in aqueous emulsion or directly. This result would be expected *a priori*, since the status of the water as the continuous phase of the emulsion is only momentary on the surface of the leaf. An aqueous spray mixture prepared with the emulsive spray oil formulation used in these experiments would be "quick breaking" (deOng *et al.* 1927). In "quick-breaking" mixtures, the oil is released and forms a film on the surface of the leaf at the moment of contact of the spray. The water is free. Most of the water runs off the leaf immediately. The remainder collects in drops and evaporates, generally within an hour in conventional spraying in citrus orchards in southern California in the late summer and early fall.

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Bucculatrix gossypiella—A Potentially Important Pest of Cotton[1]

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Bucculatrix gossypiella Morrill[3] was collected from cotton in Mexico and described as a new species by Morrill (1927a), who later reported the bionomics of this species and its distribution in Mexico (1927b). According to records in the U. S. National Museum and the Plant Pest Control Division's Survey Section, specimens have been collected from the southern district of Baja California and the States of Sonora, Sinaloa, and Tamaulipas in Mexico, and from Colombia and Peru. However, until 1957 the species had not been reported in the United States. In September of that year this insect was first noticed attacking cotton growing in the ½-acre screen cage at the Entomology Research Center at Brownsville, Texas. A month later the cotton plants had been seriously damaged by the insect.

Damage to bolls, leaves, and stems of cotton by this insect was observed. It was found that larvae attacking bolls tunnel in the carpel without penetrating through the inner wall. The inner wall turns yellow, and as a result the lint is discolored. Most of the damaged bolls show reddish brown spots about 4 sq. mm. in area. Apparently these spots are formed when pigment cells are damaged by the larvae. As a result the pigment spreads to adjacent cells and appears on the outer carpel wall. Heavily infested bolls may open prematurely, rot, or become deformed. The larvae also attack leaves by tunneling into the large veins and that portion of the petiole adajcent to the leaf blade. The damaged leaves become dark green in color and droop. Tender leaves often wilt when young stems bearing them are attacked.

In October 1957 many leaves and stems of all the cotton plants growing in the screen cage were infested by this insect, and all the bolls were heavily damaged. From 50 infested bolls collected during the month, 722 larvae emerged, averaging 14.4 larvae per boll. Ninety per cent of the larvae became adults. The larvae pupated in 2 to 3 days after cutting out of the bolls, and the pupal period was 5 to 6 days. None of these larvae or pupae were parasitized. On the basis of these observations, it was concluded that this insect can cause serious loss in both quality and yield of cotton. Field observations could not be made, because all field-grown cotton plants in the vicinity of Brownsville were destroyed before the first of September, in accordance with pink bollworm control regulations.

Table 1.—Per cent of cotton bolls infested with *Bucculatrix gossypiella* after treatments with several insecticides.[a]

INSECTICIDE	PER CENT OF BOLLS INFESTED[b]	
	Five Applications	Six Applications
Guthion	35	45
Monsanto CP-7769[c]	25	45
Chipman R-6200[d]	25	40
DDT-dieldrin	5	15
Untreated check	72	95

[a] Data obtained by W. L. Lowry, of the Entomology Research Center, Brownsville, Texas.
[b] Based on 20 bolls from each treatment and 40 untreated bolls.
[c] Hexaethyl (ethylthiomethylidyne) triphosphophosphonate.
[d] *O,O*-diethyl *S*-(2-dimethylamino)ethylphosphorothiolate, paratoluene sulfonate.

A small-scale insecticide test for control of the pink bollworm and boll weevil was carried out in the screen cage during August and September, and incidental observations were made on control of *gossypiella*. Plots 16×16 feet were sprayed with several insecticides at the rate of 0.5 pound per acre, except DDT-dieldrin mixture which was applied at 2 pounds of DDT and 0.5 pound of dieldrin per acre. Applications were made at 5- to 7-day intervals. Records of bolls infested with *gossypiella* taken after the fifth and sixth applications are summarized in table 1.

DDT-dieldrin mixture was the most effective treatment. Infested bolls from the plot receiving this treatment were very lightly damaged while those from the untreated plot were very heavily damaged.

It is not known how long *gossypiella* has existed at Brownsville. Since this insect occurs in Tamaulipas, Mexico, which borders the Rio Grande Valley of Texas, it may have been in this Valley area for some time. Clark (1929) reported that "the cotton *Bucculatrix*" appeared in the Lower Rio Grande Valley, but it is not clear whether he referred to *gossypiella* or to *B. thurberiella* Busck.

Since *gossypiella* is potentially an important pest of cotton, more information is needed to determine its distribution in the United States, and the extent of damage done to cotton in various areas.

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[3] Lyonetiidae: Lepidoptera.

DDT Injury on Red Fescues[1]

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Conspicuous leaf-reddening of long duration on plants of red fescue, *Festuca rubra* L., and Chewings fescue, *F. rubra* var. *commutata* Gaud., during April and May 1956, 1957, and 1958, in western Oregon, was caused by DDT that had been applied for control of silver top (Hardison & Krantz 1957, Hardison *et al.* 1958). Treated fields of Chewings fescue were easily distinguished at a distance by red streaks that marked the path of sprayers. Striking patterns, streaks of brighter red resulting from overlapped treatments, untreated narrow islands of normal green between treated swaths, and half circles at the edges of fields caused by turning of spray equipment, were noted in fields. Leaf-reddening was proportionally more severe where heavier rates of DDT spray were applied, and plants turned white where excessive amounts were discharged accidentally.

No apparent injury was noted during 1956 on other grasses in fields sprayed with 1 pound of the active ingredient of DDT per acre. These grasses included *Agropyron repens* (L.) Beauv., *Agrostis tenuis* Sibth., *Dactylis glomerata* L., *Elymus glaucus* Buckl., *Festuca arundinacea* Schreb., *Holcus lanatus* L., *Lolium multiflorum* Lam., *L. perenne* L., *Phleum pratense* L., *Poa compressa* L., and *P. pratensis* L. However, scorched leaves of *Holcus lanatus* occurred at sides of fields where heavy rates of DDT were discharged.

Grasses in a row nursery at Oregon City, Oregon, were sprayed May 9, 1957, at the rate of 1 pound of DDT (emulsifiable concentrate) in 27 gallons of water per acre. Conspicuous leaf-reddening resulted within 2 weeks on plants of red fescue of several varieties (Penn. F55(38), Penn. F74(38), Penn. 78(38), Pennlawn, and Illahee) and of Chewings fescue. No injury was apparent on *Agropyron trachycaulum* (Link) Malte (variety Primar), *Alopecurus pratensis* L. (Oregon 11 and 12), *Bromus arvensis* L. (commercial), *B. catharticus* Vahl (commercial), *B. inermis* Leyss. (Homesteader), *Dactylis glomerata* (S-143, P.I. 109, 072, Oregon Sel. 233, Trogdon, Minerva, and Tardus 11), *Festuca arundinacea* (Alta, Ky. 31, and Goar), *F. elatior* L. (Mimer), *Lolium multiflorum* (commercial), *L. perenne* (S-23 and commercial), *Panicum virgatum* L. (commercial), *Phalaris arundinacea* L. (Ioreed and commercial), *Phleum pratense* (Lorain and Cornell 1777), or *Poa pratensis* (Merion and commercial).

No apparent damage to Chewings fescue over a 3-year period resulted from applications of sprays during April and May at the rate of 27 gallons per acre containing several other chemicals in the following dosages, and formulations: In 1956 wettable powders: methoxychlor, 16 ounces; Karathane, 5 pounds; emulsifiable concentrates: heptachlor, 32 ounces; Kelthane, 8 ounces; and malathion, 12 ounces. In 1957 emulsifiable concentrates: aldrin, 8 and 16 ounces; dieldrin, 8 ounces; endrin, 8 and 16 ounces; and heptachlor, 4, 8, and 16 ounces. In 1958 emulsifiable concentrates: aldrin, 4, 8, and 16 ounces; and heptachlor, 4, 8, and 16 ounces. Lack of injury from these chemicals in sprays formulated from dry and liquid concentrates indicates that DDT itself is responsible for injury to Chewings fescue.

Sprays of DDT formulated from wettable powders (8, 16, and 32 ounces of actual toxicant per acre) and applied during 1956 or from emulsifiable concentrates (8 and 16 ounces of actual toxicant per acre) and applied during 1957 and 1958 caused more injury as the rate was increased. Most field and experimental sprays of DDT were applied in approximately 25 gallons of water per acre. DDT caused leaf-reddening on Chewings fescue after application in sprays at weekly intervals from mid-April to mid-May 1956 and 1957. The most effective time for application of DDT to control silver top of Chewings fescue in western Oregon was immediately prior to emergence of most inflorescences during late April, but during this period of growth injury to plants resulted from this chemical during 1956, 1957, and 1958. In a few fields of Chewings fescue in which DDT was applied during May 1957 after emergence of inflorescences from most culms, a severe scorch resulted on both leaves and panicles. Seed development in the lower portions of some of these panicles was inhibited.

During 1958 DDT applied as a 5% dust during the latter part of April and the first part of May caused leaf reddening in Chewings fescue plants in western Oregon. DDT at 1.25 pounds per acre in a dust formulation eventually caused as much apparent leaf reddening as in sprays, but the toxic action of DDT in dusts was slower than in sprays. DDT injury from sprays was evident within 2 weeks, whereas injury from the chemical in dusts was not noted until 3 weeks after treatment.

Only a few reports of DDT injury on plants of the family Gramineae were found in the literature. DDT spray was reported to scorch severely the foliage of oats (Cunliffe & Hodges 1946, p. 359), and DDT spray that drifted from peach orchards was reported to cause crabgrass (presumably *Digitaria* sp.) to turn a reddish brown but later a normal green color returned (Driggers 1946). DDT applied to velvet bentgrass, Colonial bentgrass and annual bluegrass (presumably *Agrostis canina*, *A. tenuis* and *Poa annua*, respectively) in golf greens was apparently nontoxic (Kerr 1948).

DDT was used on red fescue in Pennsylvania for silver top control, and no leaf injury was reported (Pa. Agric. Expt. Sta. 1948). However, the leaf-reddening of red and Chewings fescues by DDT during 1956, 1957, and 1958 in Oregon is regarded as sufficient evidence to caution that use of this chemical on fine-leaf fescues of the *Festuca rubra* group may result in phytotoxicity. The problem of DDT residues in treated fields usually is avoided in Oregon, because the straw and stubble in most grass seed fields are burned after harvest for disease control (Hardison 1957a, 1957b, 1958).

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Notes on the Biology of the Asiatic Oak Weevil in Maryland[1]

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The Asiatic oak weevil, *Cyrtepistomus castaneus* (Roelofs), was first reported in Maryland in September, 1949, and it has been a pest in chestnut nurseries and orchards in Maryland (Johnson, 1956). It is common where oaks are found in many parts of central and southern Maryland and on the Eastern Shore. Triplehorn (1955) found 44 species of plants on which this weevil would feed either in the field or in the laboratory. Descriptions of the four stages and feeding habits are included in his report. The adults feed on the leaves, and it has been assumed that the larvae feed on the roots.

Johnson (1956) reported that damage from the adults in Maryland generally occurs in early August and becomes progressively more severe by middle September; while Triplehorn (1955) found that in Delaware severe damage was done to oak seedlings by the adults before the end of June.

It has not been definitely known how this weevil overwinters in Maryland. The purpose of the work reported here was to study this aspect of its life cycle. Triplehorn (1955) suspected that the Asiatic oak weevil overwintered in the larval stages in Delaware. He found the first larvae about 5 to 6 inches beneath the forest litter around the roots of small white oak seedlings on March 5 near Newark. Adults were recovered from the leaf litter in midwinter, and weathered specimens as early as May 10. These apparently were of the previous year's generation, but Triplehorn did not think that these adults contributed to a new generation.

MATERIALS AND METHODS.—The following studies were made at the University of Maryland Plant Research Farm near Fairland where a large weevil population occurred.

Groups of 10 adult weevils were each placed in four glass canisters on October 20, 1957. The canister was made of a wooden frame approximately 9×11×1 inches with glass plates forming the two sides. A cross section of the soil and forest litter from the infested area, and a small oak seedling were placed in each container. The top of the canister was covered with screen, taped in place, and all openings closed with paraffin. The canister was then buried in the forest with only the top of it above the ground.

The purpose of the canister was to simulate natural conditions. It was thought that weevils might be observed through the glass, but no observations were made. The canisters were dug up at intervals during the winter. The contents were examined macroscopically for adults, and then placed in a Berlese funnel for any that might have been missed.

Three Berlese soil samples about 1 foot square and 6inches deep were taken at intervals of about 2 weeks from November 9, 1957 to July 14, 1958 from around the base of white oak seedlings in an area that had been infested with the adult Asiatic oak weevils the summer and fall of 1957. The sample included the ground litter. The purpose of this sampling was to determine the presence of larvae or adults in the soil during this period of the year.

Ten "sticky boards" were hung from limbs of trees in the infested area. These consisted of 6- ×10-inch plywood sheets painted yellow-coated with a tree banding compound sold under the trade name of "Stop." These boards were inspected at intervals of about 2 weeks from November 9, 1957 to July 14, 1958.

The first appearance of the adult weevils in the spring and summer was determined by shaking the leaves of five different oak seedlings over a white cloth at intervals of about 2 weeks from May 31 to July 14, 1958.

RESULTS.—Live adults were recovered on November 10, December 21, and February 27 from the glass canisters. No live adults were found in the container opened on April 26. Dead adults were found in all the canisters.

Asiatic oak weevil larvae were recovered from the Berlese soil samples from December 1 to June 14. From December 1 to April 26 the larvae ranged in size from 1.5 to 3.7 mm. in length. One larva recovered on June 14 was 7 mm. in length. Few larvae were recovered during May and June in comparison with the numbers found in samples taken during the previous months. No adult weevils were found in these samples.

One adult Asiatic oak weevil was recovered from a sticky board on December 1.

Adults of the Asiatic oak weevils were first found on five oak seedlings on July 14 when a total of 21 were counted. Inspection of the same area on June 28 had been negative.

On the basis of the data gathered in this study, the Asiatic oak weevil is capable of overwintering in the larval stage in Maryland. While adults were recovered alive as late as February 27, it appears that they do not survive throughout the winter in the leaf litter and soil to a depth of 6 inches nor in confinement to a depth of 10 inches. Adult Asiatic oak weevils were first recovered from the leaves of oak seedlings on July 14. Circumstantial eivdence points to a period of pupation in May and June.

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Sick Milkweed Bugs[1]

R. L. BEARD, *The Connecticut Agricultural Experiment Station, New Haven*

A peculiar, elusive, pathology of the large milkweed bug (*Oncopeltus fasciatus* (Dall.)) in culture is of dual interest. As this bug is one commonly used in the laboratory for biological and toxicological study, healthy cultures are required. On the other hand, the pathology in question appears unique in some ways and is of interest for its own sake in the study of insect diseases.

The occurrence of the malady is so sporadic and unpredictable that continued and well-planned experimentation is difficult. It can appear suddenly in one of many culture boxes, wipe out the majority of the individuals, and then disappear for weeks or months at a time. Even though bugs have been reared in rooms with constant temperature and some degree of humidity control, the pathology has seemed to develop more frequently during late summer and during periods of high humidity. Notable exceptions have occurred, however, and bugs reared in high humidity have not succumbed. In most cases, affected bugs have been living under crowded conditions, but this, too, is not invariable. Also, overcrowding frequently occurs with no hint of the illness. There has been no consistent pattern attributable to genetic control. For example, in 66 segregated lines of bugs, the incidence of the malady was infrequent and could be associated with no particular line nor type of selection.

Within almost every affected culture some bugs survive, show no symptoms of the ailment, are able to reproduce normally, and their progeny are healthy. These surviving bugs escape the malady, have a real immunity or tolerance, or recover from undetected mild attacks.

The affliction characteristically strikes the nymphs in the

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fourth and fifth instars. Few third instar bugs are affected, and a healthy bug in late fifth instar will probably survive.

The general effect suggests an upset water metabolism. A discoloration is the first sign of illness, giving the bug a water-soaked appearance. There is usually diarrhea and always an especially characteristic, offensive, odor. Sick bugs gradually become inactive and shrink in size dorso-ventrally with a loss of body fluids. At times a temporary swelling precedes the deflated condition. If affected bugs try to molt, they die in the process as if moisture were inadequate. Fresh smears of body tissues show no obvious microparasites nor do they suggest the nature of the pathology. Sectioned tissues show compaction, rather than disintegration, as if desiccation was involved. Bugs showing definite symptoms do not recover even when isolated and given a clean environment with fresh food and water.

If an infection is involved, the causal agent and its means of transmission are not known. No microorganisms have been detected, and a virus infection is unlikely. All attempts to induce the infection have failed. Healthy bugs can tolerate without ill effects injections of water extracts of sick bugs and water extracts of food material contaminated by affected bugs. Healthy bugs can actually feed on sick bugs without becoming ill. The possibility was considered that the bugs acquire infection early in life without showing symptoms until the fourth instar is reached. Accordingly, eggs were placed in dirty boxes with old food materials which had harbored large numbers of sick bugs and in which the foul odor persisted. A bare minimum of fresh food was supplied. The eggs hatched and nymphs developed with no sign of the pathological condition.

If this is not an infection, several possible explanations are suggested. One is that this is a peculiar metabolic condition induced by a combination of certain environmental factors at present unknown. The epizootic aspect could appear because the inducing conditions might affect many bugs at the same critical time. Another possibility is that although microorganisms may not infect the bugs, toxic substances could be liberated by microorganisms growing in the food. Or, elements in the food itself (dried milkweed seeds) could be toxic. If so, the pathology is more like a chemical poisoning than a disease in the usual sense. It might also be considered that a virus- or Salmonella-type of infection causes such a severe, acute, enteritis with consequent dehydration that recovery is not possible. These are speculations at present, but they suggest possibilities to be explored if the occurrence of the ailment permits further study.

Effectiveness of Two Carbamates against DDT- and Malathion-Resistant House Flies[1]

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Resistance of house flies (*Musca domestica* L.) to chlorinated hydrocarbon insecticides is now world-wide, and resistance to many organophosphorus compounds is also developing. In investigations of compounds in other chemical groups certain carbamates have shown promise as possible substitutes for house fly control. Tests were conducted with two carbamates to determine the extent of cross-resistance to these compounds in DDT- and malathion-resistant flies. Topical applications of 4-methyl-2-propylpyrimidyl *N,N*-dimethylcarbamate (hereafter called methyl carbamate), dimethyl 5-(3-methyl-1-phenyl-pyrazolyl) carbamate (Pyrolan), malathion, and DDT were made to flies of three colonies—the Grothe colony, highly resistant to malathion and moderatedly resistant to DDT; the No. 1 colony, completely resistant to DDT and slightly resistant to malathion; and the regular colony, susceptible to both DDT and malathion.

The applications were made with a microsyringe, which consisted of a ¼-ml. hypodermic syringe with a No. 27 needle, mounted on the frame of a microtome. The mechanism of the microtome was used to advance the plunger of the syringe and the volume of solution delivered was controlled accurately in doses varying by 0.1 microliter. Female house flies were anesthetized with carbon dioxide, weighed individually, and divided into classes according to weight. Each class contained flies within 1 mg. of the average weight, *e.g.*, 18 ± 1 mg., 20 ± 1 mg., etc. The volume of the solution applied was varied in accordance with the weight of the flies. so that the rate of application remained constant at 0.1 microliter per 2 mg. of body weight. The insecticides were applied in acetone solutions at concentrations that gave a range of mortality after 24 hours. From 20 to 40 flies were used at each concentration. The results are given in table 1.

Against regular flies the methyl carbamate was slightly more effective than DDT or malathion, whereas Pyrolan was less effective. Both carbamates were more effective than DDT or malathion against Grothe colony flies; resistance in this colony was about 400-fold to malathion, 40-fold to DDT, and 8- to 10-fold to the carbamates. Both carbamates were more effective

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Table 1.—Per cent mortality of three strains of house flies treated with topical applications of DDT, malathion, and two carbamates.[a]

Concentration (%)	DDT			Malathion			4-Methyl-2-propylpyrimidyl *N,N*-dimethyl carbamate			Pyrolan		
	Grothe	No. 1	Regular	Grothe	No. 1	Regular	Grothe	No. 1	Regular	Grothe	No. 1	Regular
40.0	—	0	—	100	—	—	—	—	—	—	—	—
20.0	—	—	—	85	—	—	—	—	—	—	—	—
15.0	—	—	—	65	—	—	—	—	—	—	—	—
10.0	—	—	—	15	—	—	—	—	—	100	—	—
5.0	80	—	—	—	—	—	—	—	—	100	90	100
2.5	60	—	—	—	—	—	—	—	—	90	90	—
1.0	25	—	—	—	100	—	100	—	—	35	25	100
0.75	—	—	—	—	100	—	—	—	—	—	—	—
.5	5	—	—	—	95	—	100	100	—	—	15	100
.25	0	—	90	—	90	—	90	100	—	—	—	80
.1	—	—	—	—	5	100	15	10	100	5	5	0
.075	—	—	80	—	—	100	—	—	—	—	—	—
.05	—	—	—	—	10	75	5	0	100	—	0	—
.025	—	—	45	—	5	10	—	—	70	—	—	—
.01	—	—	3	—	—	—	0	—	0	0	—	—

[a] 2 to 4 replications of 10 females each.

than DDT against No. 1 colony flies, and the methyl carbamate was slightly more effective than malathion; the resistance shown by this colony was complete to DDT, about 5-fold to malathion and 8- to 10-fold to the carbamates.

The resistance of the Grothe and No. 1 flies to the carbamates suggests that these compounds might not be effective in some areas, and might not remain effective long in others. It is interesting however to note that the highly malathion-resistant Grothe colony showed no more resistance to the carbamates than the No. 1 colony, although malathion and the carbamates are both cholinesterase inhibitors.

Movement of Flower Thrips through Greenhouse Ventilators[1]

THOMAS J. HENNEBERRY, FLOYD F. SMITH, and EDGAR A. TAYLOR, *Entomology Research Division, Agric. Res. Serv., U.S.D.A.*

Heavy migrations of flower thrips (*Frankliniella tritici* (Fitch)) occur each spring and summer throughout the eastern United States. Trapping records by means of sticky cards placed on a 21-foot tower and a steeple showed that these thrips move through the air in approximately equal numbers at different heights up to 135 feet (Taylor *et al.* 1956). During their 4- to 6-week migration period the thrips damage roses, peonies, iris, hemerocallis, and many other garden flowers. They also enter greenhouses through the ventilators and severely damage light-colored flowers, especially roses, carnations, and chrysanthemums.

Karlin *et al.* (1957) reported highly successful control of these thrips in greenhouses by screening both side and top ventilator openings with dieldrin-impregnated cheesecloth. They also obtained control when only the side ventilators were screened.

In experiments conducted in 1953 at our Beltsville, Maryland, laboratory greenhouses, roses and chrysanthemums were protected from thrips injury by covering the side ventilators with aster cloth or nylon screen and spraying it weekly with an emulsion or suspension containing ¼ pound of dieldrin or heptachlor per 100 gallons. Similar control has been obtained in nearby commercial greenhouses. Malathion aerosols were applied every 7 to 10 days inside the greenhouse to destroy the thrips present. The failure of similar malathion treatments to control thrips in unscreened greenhouses indicated that they were entering through side ventilators.

During the summer of 1957 and 1958 trapping records were made in a greenhouse to determine the movement of thrips through side and top ventilators. The prevailing wind each season was from west to east.

In 1957 trap cards 2 by 5 inches, coated on one side with a sticker (Deadline) were placed at 18-foot intervals in the ventilator openings along an empty 75-foot greenhouse at the Beltsville Agricultural Research Center. The cards were placed in the top and the side ventilators on both the west and east sides of the greenhouse, one facing outward to catch the thrips as they entered and the other facing into the greenhouse to catch those leaving. From June 4 to July 17 five cards at each position were exposed for 1 week; then they were taken down and examined for thrips and replaced with freshly coated cards. In 1958 potted roses were grown in the greenhouse, and the trap cards were placed every 9 feet along the ventilators. From June 5 to July 16 nine cards were similarly exposed at each position.

Table 1.—Total numbers of flower thrips trapped entering and leaving greenhouse ventilators during the seasons of 1957 and 1958.

Trap Location	1957		1958	
	Entering	Leaving	Entering	Leaving
Top				
East	2	32	5	87
West	11	5	73	6
Bottom				
East	2	2	7	14
West	8	10	27	14
L.S.D. (19:1)	10		24	

Table 1 shows the numbers of thrips captured on all cards for each of the two seasons. More thrips were caught in the top ventilators than in the side ones. Also in the top ventilators most of the trapped thrips were either entering the greenhouse on the west side with the prevailing wind or leaving on the opposite side. There were as many caught leaving as entering, an indication that most of those entering the greenhouse through the top ventilators leave through the ventilators on the opposite side aided by wind currents and by convection due to heated air from within the greenhouse. It appears that screens in the top ventilators are of little value.

Although fewer thrips were trapped entering the side than the top ventilators, few were carried out of the greenhouse through the side ventilators on the opposite side. The distance between these ventilators is nearly 18 times as great as in the top, but probably most important is the lack of convection currents. Thus most of the thrips entering the greenhouse through the side ventilators apparently remain to infest and damage the crop therein. These data support the practice of screening only side ventilators for thrips control in the greenhouse.

A study was also made to determine whether thrips were able to pass through wet evaporative-type excelsior pads through which air is drawn by fans, a recently developed system for cooling greenhouses in the hot summer months. Coated trap cards were set up about an inch from the inside and outside surfaces of an installed cooling pad to catch thrips approaching it from outside the greenhouse and also those passing through. On eight cards that were in each position for 1 month, 13 thrips were caught outside the greenhouse and 31 inside, which apparently passed through the cooling pad. Dust accumulation may have made the outside traps less efficient. These limited tests support observations in commercial houses that thrips as well as other insects gain access to greenhouse crops through the cooling pads.

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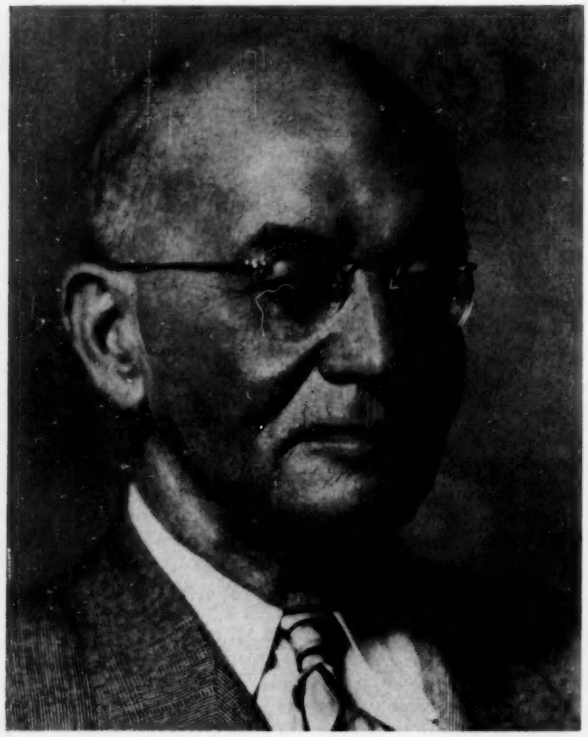
[1] Accepted for publication October 15, 1953.

OBITUARIES

Frederick B. LaForge
1882-1958

Dr. Frederick B. LaForge, 76, well known chemist for many years in the U. S. Department of Agriculture, died September 4 in Washington, D. C. after a long illness.

Born in Bridgeport, Conn., on March 12, 1882, Dr. LaForge graduated from Princeton University in 1905 and received the A.M. and Ph.D. degrees from Berlin University in 1908. After completing the work for his doctorate he assisted Prof. R. Pschorr in the investigation of opium alkaloids at Berlin University. Upon returning to America he served from 1910 to 1915 as Assistant and Associate Fellow in the Rockefeller Institute for Medical Research, where he carried out studies on nucleic acids, sugars, and other biological products.



Frederick B. LaForge
1882–1958

In 1915 Dr. LaForge entered the U. S. Department of Agriculture. There he began the investigation of rare sugars. He was the first to find a 7-carbon sugar in nature, a discovery that proved important in connection with the synthesis of carbohydrates. He developed and patented a method for the production of furfural from corn cobs. In 1927, when the Division of Insecticide Investigations was established in the Department, Dr. LaForge started intensive studies on insecticides of plant origin. In 1933 he received the Hillebrand award from the Chemical Society of Washington as co-discoverer with Dr. H. L. Haller of the molecular structure of the important insecticide rotenone. Following this he investigated the chemistry of pyrethrum, one of the most widely used and safest insecticides. He contributed much to our knowledge of the chemical structure of its active constituents. This work culminated in 1948 in the announcement by Dr. LaForge and his co-workers, N. S. Schechtor and Nathan Green, of a method for the synthesis of pyrethrin-like esters. One of these synthetic esters, allethrin, is now extensively used in insecticidal aerosols and sprays. Produced from raw materials available in the United States, it has to a large extent relieved this country of dependence on foreign-grown pyrethrum. This achievement received acclaim from chemists and entomologists throughout the world. It was recognized by a Superior Service Award from the Department of Agriculture and an Achievement Award from the Chemical Specialties Manufacturers Association.

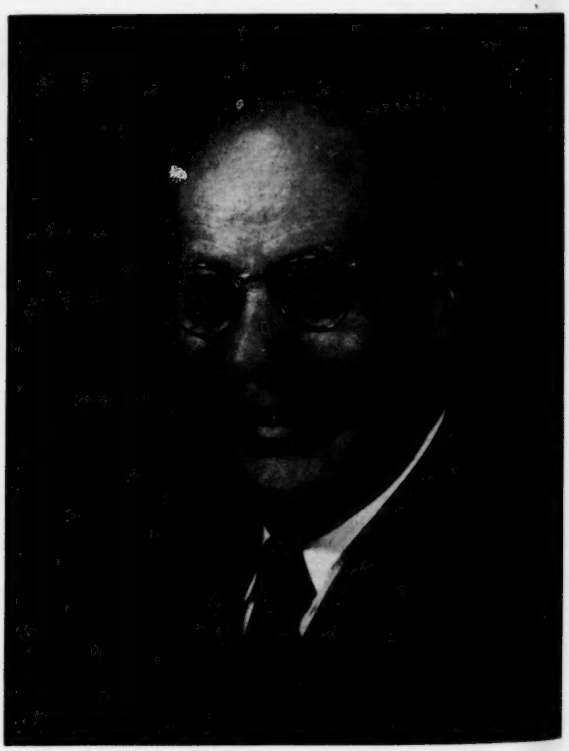
Dr. LaForge officially retired from the government service in March 1952 at the age of 70, but with typical enthusiasm for his work he continued his activities in the Department laboratory for some time afterward. He was the author of more than 100 papers dealing with the chemistry of complex materials and obtained a number of patents in the field of organic chemistry.

He is survived by his wife, Caroline, of the home address, 1509 N. 17th Street, Arlington, Va., and two sisters in Bridgeport, Conn. His consuming interest in his scientific work and his constant devotion to it will long be remembered by his associates.

RUTH L. BUSBEY

Harold S. McConnell
1893-1958

Harold Sloan McConnell was born on December 30, 1893 at Anderson, South Carolina, the son of James N. S. and Frances C. Duckworth McConnell. His boyhood was spent on the farm near Anderson which is still in the possession of the family. He graduated from Clemson Agricultural College in 1916 and came to



Harold S. McConnell
1893–1958

Maryland State College as an assistant in entomology the same year. He began work with E. N. Cory on insect pests of apples and in promoting fruit production as part of the war effort. His first publication, in collaboration with E. N. Cory, was an Extension Bulletin entitled Insects and Rodents Injurious to Stored Products. He was elected to membership in the Entomological Society of Washington at the 303rd meeting on April 5, 1917.

In January 1918, McConnell enlisted in the Army Medical Corps. He studied at Walter Reed Hospital, The Rockefeller Foundation in New York, and in New Haven, Connecticut, prior to being commissioned in the Sanitary Corps. He served in the American Expeditionary Force in Europe in August 1918 to June 1919 as water supply investigator. Upon his discharge he served as research assistant entomologist at the South Carolina Experiment Station until 1924. South Carolina Circular 31 states "H. S. McConnell, an entomologist of some years experience, was employed as assistant entomologist to conduct field plat work in Dillon, Dorchester, Barnwell and Clarendon Counties." Several publications of the South Carolina Station on the boll weevil were based largely on McConnell's work.

In July 1924, McConnell returned to the University of Maryland and resumed his research work on fruit insects. He was married to Pearl Anderson, herself a zoologist and entomologist, in 1926. There were numerous publications on oriental fruit moth and other fruit insects between then and 1942. In 1932 he received the Master of Science degree, and the title of his thesis was "Parasites of the Strawberry Leaf Roller *Ancylis comptana* Forel in Maryland."

Professionally Harold McConnell will be best remembered for his work on scale insects. His interest in this group was aroused by Philip Garman about 1921 at which time MacGillivary's "The Coccidae" was published. In 1939 he initiated a course in coccidology for graduate students. This course was taught not more than twice. It involved the techniques of slide preparation as a prerequisite to knowledge of scale insects. Because of the limited time available the students could only "scratch the surface," but they received an appreciation of the immense amount of preparatorial work necessary for scale insect studies.

McConnell's first taxonomic paper on the Coccidae appeared in 1941. There were several outstanding publications, primarily Maryland Agricultural Experiment Station Bulletin A-75 on the family Aclerdidae and Bulletin A-84 on the Planococcini in collaboration with a student, Y. M. Ezzat.

McConnell re-entered the Army Sanitary Corps during the Second World War and served as an entomologist from March 1943 to November 1945. Following the Second World War he devoted considerable time to European corn borer investigations. For 10 years he conducted an annual population survey and also distributed corn borer parasites and studied their effectiveness. McConnell devoted much time to studies of the green peach aphid on tobacco, and he developed improved methods for control of tobacco hornworms.

His membership in the Entomological Society of Washington presumably lapsed after 1917, and he was elected to membership again on February 4, 1932. He became a member of the American Association of Economic Entomologists in 1928. In 1947 he served as secretary-treasurer of the Association and business manager of the Journal of Economic Entomology.

Those closely associated with McConnell immediately acquired great respect for him primarily because of his vast fund of knowledge and his willingness to put it to use in assisting others. He was extremely well versed in systematic botany and in French, German, and Latin. He was a highly competent photographer and taught many others various techniques. He identified quickly almost any insect that was brought to him. Few entomologists know as much entomology as he did.

McConnell's outstanding characteristic was his meticulous attention to details. He was a perfectionist. This attribute was a handicap in that it meant that his research work proceeded slowly. Nevertheless his painstaking efforts are clearly reflected in his publications, particularly the taxonomic studies. His contributions as an entomologist will be long remembered, and those who knew him will miss him as a friend and adviser.

He died of cancer on May 11, 1958 and is survived by his wife and two sons, Harold M. and James F.

THEO. L. BISSELL
WM. E. BICKLEY

Thomas Roscoe Chamberlin, Sr.
1889-1958

On August 31, 1958, death as it must to all men closed the life and career of Thomas R. Chamberlin, for many years an active worker in the field of economic entomology and long-time member of this Society. His passing will be mourned by many—his family and numerous life-long friends both in and out of his profession. Thomas R. Chamberlin was a man of greatest personal integrity and charm—fearless in advocating and promoting the truth as he saw it; just and understanding in his dealings with his fellow man; warmly and fiercely loyal to his ideals. He was a "whole man"—neither so wrapped up in his professional interests as to neglect the virtues of the humanist—and yet fully aware of the overall objectives and importance of his profession, its role and its obligations.

Thomas Roscoe Chamberlin was born March 30, 1889, in Salt Lake City of pioneer Mormon stock, both his father, Thomas R. Chamberlin, and his mother, Susanna Alston, having also been born in Salt Lake City shortly following its first settlement.

His schooling at the University of Utah was interrupted in 1913 by financial problems and it was not until 1920 that he obtained his A.B. degree. He started his professional career as staff member of the Salt Lake City field station of the U. S. Department of Agriculture Bureau of Entomology in association with George I. Reeves and Cecil W. Creel, working principally on the biology and control of the alfalfa weevil with special reference to its parasites. In 1921–23 he was stationed at Hyères in southern France to investigate and collect parasites of the alfalfa weevil for introduction into the United States. This work was highly successful and a number of parasites were introduced which aided greatly in bringing this important insect under control. Following the completion of this assignment he returned to Salt Lake City where he continued his work on the alfalfa weevil until 1926, at which time he was transferred to Forest Grove, Oregon, where he worked in association with the late Lawrence P. Rockwood on wheat insects—principally the wheat joint worm and the wheat stem maggot. He found this environment and association so warm and friendly that he ever after felt this to be his true home —the one to which he would ultimately return. He was called in 1935 to Madison, Wisconsin, where he was in charge of extensive researches on white grubs. Here he formed many of his finest associations under congenial and pleasant working conditions.

In 1943 he was again transferred, this time to State College, Starkville, Mississippi, where he conducted work on corn insects, principally the corn earworm and fall armyworm. At his own request, he was reassigned in 1946 to work at Madison, Wisconsin where he completed his professional career in studies of various legume and grass insects, including the meadow spittlebug. On October 31, 1950, he retired after 37 years' continuous service in the U. S. Department of Agriculture Bureau of Entomology and succeeding services. At this time he returned to Forest Grove and established the home he continued to occupy until his death.

Thomas R. Chamberlin's life was a rich and happy one and the years of his retirement were especially so—rich in leisure to serve and enjoy his family and friends. He was married in Salt Lake City September 2, 1913. His wife, née Reta V. Folland, survives him as do his three children (a son, Thomas R. Chamberlin, Jr., of Newport, Oregon, and two daughters, Mrs. Miriam Jean McKee of Forest Grove, Oregon, and Mrs. Joanne McTaggart of Madison, Wisconsin) and twelve grandchildren.

Thomas R. Chamberlin, Sr. was a member of the honorary scientific societies Sigma Xi and Phi Delta Theta, and was an active member of the American Association for the Advancement of Science; the Entomological Society of America; the Washington Entomological Society; and the Oregon Entomological Society.

He was not an especially prolific writer but was the author or co-author of more than 20 papers on a variety of entomological subjects, including two U. S. Departmental Technical Bulletins; one circular; an Oregon State Bulletin; and a number of "Journal" articles—concerning the alfalfa weevil and its parasites; the wheat jointworm and wheat stem-maggot; sweet clover weevil; spotted cucumber beetle; corn earworm, fall armyworm, June beetles; Ladino clover seed pests, spittlebugs and grasshoppers on forage crops.

His professional work, characterized by both accuracy and conciseness, was held in high esteem by coworkers in his chosen field. It is perhaps, however, in his role as a personal friend, colleague and counseler that he reached his maximum stature—a man of simple tastes and complete integrity in harmony both with life and destiny.

JOSEPH C. CHAMBERLIN

Altus L. Quaintance
1870-1958

Altus L. Quaintance, who during the first third of this century was an outstanding figure in the economic entomology of this country, died at his home near Silver Spring, Md., on August 7, 1958.

Dr. Quaintance was born in Iowa on December 19, 1870. He received a B.S. from the Florida Agricultural College (which a few years later became the University of Florida) in 1893. He did graduate work at the Alabama Polytechnic Institute, receiving his M.S. in 1894. The same institution conferred the degree of Sc.D. on him in 1915. From 1894 through 1902 he served successively as instructor in biology and professor of entomology at the University of Florida; biologist and horticulturist of the Georgia Agricultural Experiment Station; and as zoologist, entomologist, and State entomologist at Maryland. In 1903 he received an appointment in the then Division of Entomology, U.S.D.A. Shortly thereafter he became entomologist in charge of the Division of Deciduous Fruit Insect Investigations in the newly formed Bureau of Entomology, a position that he held until his retirement in 1930. He also served as associate chief of the Bureau from 1924 to 1930, and for a number of years as a member of the Insecticide and Fungicide Board (forerunner of the present Pesticide Regulation Section, Plant Pest Control Division).

Dr. Quaintance's work dealt chiefly with insects affecting deciduous fruits. He was also a world authority on the taxonomy of the Aleyrodidae, until administrative affairs required all of his attention.

During the early part of his career Dr. Quaintance was active in professional societies. His name appears on the list of members of the American Association of Economic Entomologists for 1898; he served on many committees, and was President in 1904. He was a charter member of the former Entomological Society of America. He was also active in local organizations, including the Entomological Society of Washington, the Biological Society of Washington, and the Washington Academy of Science.

Dr. Quaintance, or "Q," as he was known to his associates, had an uncanny understanding of human nature and behavior, and an unusual ability to look ahead and visualize the probable effects of any given course of action. In spite of the importance of the various positions that he held, he was a modest, unassuming individual who never worried about personal credit. When he started his career it was customary for the head of an organization to be senior author of publications reporting research carried on by members of his staff. However, "Q" would rarely permit his name to appear as an author of a research paper unless he had actually participated in the work, or on a popular paper unless he had had some part in its preparation. In one case well known to the writer, a suggestion that he add his name to a paper reporting important research that he had outlined in detail was dismissed with a wave of the hand and the remark that "That's what I'm here for."

At the close of 1930 Dr. Quaintance retired to his farm north of Washington, to which he had given the unique name "Quaint Acres." Here he devoted his time to the production of fruit and ornamental nursery stock. Hundreds of Washingtonians stopped at his place on their Sunday afternoon drives, to buy peaches or apples if in season, ornamental plants, or some of the high-quality apple cider which was locally quite famous.

The nursery is still operated by the next generation of Quaintances; the orchard has become a thriving residential district still known as "Quaint Acres." To the younger generation of entomologists he is a shadowy figure in the early history of the profession. Those of us who knew him think of him as an extremely able scientist and administrator, who for about 30 years exerted a potent influence on the development of our profession.

B. A. PORTER

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Each manuscript submitted for publication in the JOURNAL OF ECONOMIC ENTOMOLOGY must be accompanied by an abstract suitable for publication, and prepared in accordance with instructions for the preparation of abstracts as required by Biological Abstracts. This abstract will replace the usual author's summary. See "Important Notice to Authors" which appeared in the Bulletin of the Entomological Society of America 3(2): 1. June, 1957

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